Summary and Implications of
A Preliminary Assessment of Research and Development Needs and Gaps Relevant to Bovine Spongiform Encephalopathy (BSE)

An Alberta Perspective

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Introduction

This report was developed under the direction of the Alberta Science and Research Authority together with the Alberta Agricultural Research Institute and Alberta Agriculture, Food and Rural Development. Conducting an assessment of the global situation for Bovine Spongiform Encephalopathy (BSE) research and identifying areas where Alberta's scientific capacity can be best utilized and identifying where new related R&D endeavors should be initiated, were the key outcomes identified for the first phase of the Prion Sciences Initiative. This report provides a brief background to the global BSE situation, identifies the R&D priorities and implications for Alberta, and describes the knowledge and emerging research challenges that exist.

Background

Enhancing research capacity in BSE is but one of a number of strategies directed toward elimination of this disease. The global R&D capacity in BSE science is gaining momentum and is strongly linked with research on other Transmissible Spongiform Encephalopathies (TSE) and their impact on human and animal health.

BSE was first recognized in the United Kingdom in 1986 and drew significant public attention around the world when in 1996, it was linked with a human disease – Creutzfeldt-Jakob Disease (CJD). BSE then challenged the continental European agricultural, food and human health sectors as detection of the disease became more frequent. Increased risk analysis, surveillance and control measures based on a growing body of knowledge have reduced the epidemic significantly, and have created hope for a speedy elimination of BSE. The recent detection of BSE in North America has created challenges to this continent's livestock industry by diminishing public confidence in both food safety and science's ability to eliminate any and all potential risks to humans.

The application of basic and applied sciences through knowledgeable animal and human health expertise has directly resulted in the decreasing prevalence of BSE globally. There are still many remaining outstanding issues related to BSE and TSEs that require new knowledge to ensure the long-term elimination of these diseases. Progress on BSE and other TSEs may create applicable knowledge to other animal and human diseases, as better detection and clarity of how infective agents create diseases are uncovered.

Alberta has a globally-focused beef and dairy industry. The detection of the one case of BSE in Alberta has created impacts and challenges that parallel those experienced by the 14 jurisdictions where BSE has been detected. Alberta has committed to identify and address the needs for enhancing R&D efforts to sustain progress toward eliminating and better understanding BSE. These efforts need to be linked with the global initiatives on BSE and it is essential that each jurisdiction choose areas to build new science capacity utilizing collaboration and partnerships that are multi-disciplinary and multi-institutional. Working together, it will be possible to overcome the numerous challenges that BSE and other TSEs have created.

Alberta R&D Implications

The challenges facing Alberta relate to measures taken to control the potential impacts of BSE on human health and the resultant adverse economic impacts on the cattle industry. **World trade policy discussions at the OIE and other forums hold promise for protecting human health while greatly reducing trade restrictions in a short time frame.**

Research and development has been ongoing on many aspects of TSEs for many years and on BSE since its discovery in 1986. These diseases are very difficult to understand and research progress has been hard won.

Testing of cattle to remove BSE positive animals from the food and feed systems currently relies on testing of the brain tissue collected at slaughter or after death. The three testing methods used in Canada were developed through R&D conducted in the UK and France. Because of the high cost of testing all cattle at slaughter, certain parts of the carcass are prohibited from entering the food system. As well, restrictions on the use of meat and bone meal have resulted in much lower market values for rendered products, with significant costs to the packing and feed industries. These measures were adopted in accordance with research conducted mainly in the UK.

Testing of live animals would provide a much better tool for eradication of BSE if positive animals could be reliably detected soon after infection and the test did not result in false positives or false negatives. Testing of MBM for BSE with reliable results could prevent much of the loss currently experienced.

R&D on live animal testing is being done in several countries with over 100 companies and agencies involved. Many millions of dollars have been invested over the last 10 years. Current investment in this area has been substantially increased by the USA. **Alberta could benefit by tracking the progress of current global R & D and investing in the evaluation of promising tests through programs in the EU, primarily in the UK.** New R&D in testing technologies that might be started by Alberta investment would be at a marked disadvantage compared to competing programs that are well advanced. The routine use of live animal testing is likely several years away as approval of governmental agencies internationally is required after suitable tests are developed.

Research is also ongoing in several countries on methods of detecting and deactivating BSE infectivity in biological materials more rapidly and effectively. Research into effective means of inactivation or decontamination is slow because current tests to prove removal of infectivity can take up to two years. R&D in this area is mainly being conducted in the EU and has been ongoing for about 10 years. **This area may have potential for Alberta investment, but there is little current scientific capacity present in Alberta**. Alberta does have R&D capacity in composting technologies that could be utilized for prion deactivation through composting. CFIA is also active in conducting composting research.

Alberta also has R&D activity relating to potential alternative uses for MBM, which is a possible solution to produce value from the product if feeding MBM is prohibited. R&D in this area will be long-term due to the time period required to prove removal of BSE infectivity from potential fertilizer byproducts.

R&D related to prion inactivation is important globally in medical and research applications in order to effectively remove potential infectivity from instruments and research facilities.

Basic research is required to understand normal prion protein functions, the misfolding process, replication, molecular structure, infectivity mechanisms, interruption of the normal cellular destruction of misfolded prion protein and other topics that have the potential for creating a greater understanding of BSE and other TSEs. The primary purpose of this basic research is to develop new ways to prevent, detect, control, treat, deactivate and eradicate TSEs. Research in these topics is being pursued in several countries.

Alberta has the research capacity in genomics, proteomics, biochemistry and bioinformatics at its three universities that can contribute to the basic research gaps identified. Increased investment in expertise and facilities in these areas could provide substantial new knowledge that could result in the eventual eradication of BSE and provide key insights into other TSEs and other human brain diseases that have the protein misfolding characteristic.

Alberta and Canada have considerable expertise in epidemiology and risk management, which, in combination with the diagnostic systems, could be increasingly applied to solve short-term issues in BSE management.

Collaboration with researchers in other countries is essential to making significant progress in BSE R&D as teamwork should speed progress and avoid unnecessary duplication of efforts. For many aspects of BSE research, access to infected tissues and body fluids is necessary. These tissues and fluids must be handled in bio- containment laboratories in Canada and few of these facilities are available for this purpose. Access to infected tissue could be obtained through collaborative research with agencies in other countries, particularly the UK. In some research projects, collaboration will prove to be the only option to study BSE.

Alberta's expertise across all jurisdictions related to animal sciences and animal health should be thoroughly reviewed with respect to the emerging challenges facing the livestock industry. The total value, contribution and sustainability of the livestock industry requires advances on a number off fronts that can in part be addressed through significant science and technology investments. The public resources located at Lethbridge (AAFC, ADRI), Lacombe

(AAFC), Edmonton (UofA, AAFRD), Calgary (UofC), Vegreville (ARC) and linkage to WCVM and VIDO in Saskatoon represent a sound resource base. The potential of enhancing this capacity with Alberta's existing capacity in genomics/proteomics and novel diagnostics could create a critical mass capable of attractive leading-edge scientists, new scientific endeavor and global animal and human health commercial interests. Leadership providing strategic focus and direction in these areas of prion sciences would complement Alberta's future direction in life sciences.

A national strategy for BSE research and development would help ensure focus on the priority research gaps most critical to Alberta and Canada.

BSE Knowledge and Emerging Research Challenges

A vast array of programs, experiments and scientific publications have contributed to our understanding of BSE, yet many questions remain unanswered. The text that follows summarizes our state of knowledge and impending research challenges in six broad areas of inquiry:

- **basic prion science** – application of molecular biology, bio-chemistry, physics, etc.
- **etiology and pathogenesis** – cause and development of BSE
- **diagnostics and testing** – postmortem and antemortem or live animal testing
- **epidemiology and surveillance** – disease incidence, distribution and monitoring
- **control, prevention, therapeutics and vaccines** – treatment and preventative measures
- **inactivation and decontamination** – destruction of prions in the natural environment and laboratories.

Basic prion science

The scientific community has identified prions (i.e., infected or abnormally folded prion proteins) as a central cause of BSE. The degree of consensus and understanding is substantially less regarding the contributing factors and processes that convert normal prion protein (PrP^c) present in all mammals to its infectious state: the prion (PrP^{res}). The normal soluble prion protein is somehow misfolded into an insoluble protein that accumulates in cells and is not recognized as abnormal by the immune system. Many scientists cite the work of Nobel Prize winner, Dr. Stanley Prusiner, as proof that prion protein conversion is a protein-protein replication phenomenon. However, prion research over the past decade has pointed to other possible contributing factors such as RNA molecules that may stimulate the conversion process.

Effective prion research occurs at the intersection of the domains and sub-domains of biology, chemistry and more recently, physics. Many prion researchers report a lack of collaborative approaches on account of individualized research agendas. This is understandable, in part, due to the early stage of prion R&D and the numerous experiments that are being conducted globally. The lack of major scientific breakthroughs in the field of prion research has, however, provided time and impetus for broader, goal-oriented research funding initiatives to take root at the national level in both the United States and the European Union (EU). As the relevant disciplines and strategies converge and increasingly sophisticated and powerful equipment and analytical methodologies become available, basic prion laboratory research is still constrained on account of strict bio-containment requirements. The declining occurrence of vCJD also diminishes the political pressures for future research funding in the UK.

Twenty years of prion research has helped identify that the causative agents of TSEs are not viruses, bacteria or parasites. The novel nature of prion diseases has made progress slow and created controversy. Basic prion research has resulted in the identification of TSEs as well as over 20 strains of spongiform encephalopathy for sheep and approximately 10 different strains for humans. Apart from the possible identification of new strains of BSE in Japan and Italy, only one strain has been confirmed for BSE in cattle thus far. Genetic research has recently identified potential contributing factors to prion folding and may lead to the creation of BSE-resistant animals. Genetic work in identifying surrogate markers may be foundational in the development of an acceptable diagnostic test for BSE. Work in the area of molecular biology is beginning to elucidate the normal functions of prion proteins and potential factors involved in the misfolding process. While additional research is required, structural aspects of prions are becoming clearer through application of electronmicroscopy, mathematical and computer modeling and divergent fields such as structural biology, neuropathology and nuclear physics.

Basic prion research contributes to applied research work in pathogenesis, therapeutics and diagnostics. Basic research can either be foundational to discovery in applied areas of research or the place where breakthrough discoveries themselves occur. The fact that research groups have been active in this and other areas of BSE-related research for the past 15 to 20 years bears consideration in formulation of future research work.

Emerging Research Challenges in Basic Prion Research

Global

- ✓ clarification of the nature of the agent and identification of those structural features and their potential for variation
- ✓ determination of the function of prion protein and clarification of role where present
- ✓ determination of the mechanism of conversion to pathogenicity and replication
- ✓ pathogenesis events and mechanisms of prion dysfunction
- ✓ extension of the molecular genetic studies of the PrP gene (and others identified as associated with TSE) in cattle (and sheep)
- ✓ identify the extent of polymorphisms in the PrP gene of humans and animals to identify genetic associations among distantly related species
- ✓ development of live animal models

Local

- ✓ strengths in proteomics, genomics, micro-fluidics and bio-informatics, potentially applicable to advancing the knowledge of BSE and other TSEs
- ✓ potential to build partnership with The Synchrotron Institute in Saskatoon to better understand protein structure and folding
- ✓ Level 3 laboratory facility in Lethbridge (CFIA lab) appears to be under-utilized
- ✓ AAFRD and University of Alberta have proposed the construction of Level 3 facilities
- ✓ diagnostic, research and regulatory functions critical to respond to the directions from the International Expert Panels on BSE (recent Canada/US panel included) are available in Alberta
- ✓ Alberta has recognized diagnostic and testing capability for BSE and CWD
- ✓ Alberta has recognized research expertise in epidemiology, diagnostics and testing methodologies that can and does collaborate with researchers and scientists in other locations in Canada, the UK, Japan and the US

Etiology and Pathogenesis

BSE may have originated from a spontaneous genetic mutation in cattle, from scrapie in sheep (this hypothesis is losing merit in light of more recent research) or from TSE in another animal species. In spite of this uncertainty, we are increasingly aware that very small doses of infective material in feed can lead to BSE infection. While other hypotheses on the origin of BSE point to copper deficiency or exposure to organophosphates, the Harvard Centre for Risk Analysis points out that epidemiological data does not support these hypotheses.

The Office International des Epizooties (OIE) reports in its 2003 Technical Scientific Review of prion diseases in animals that "*The role of the prion protein in the pathogenesis of these neurodegenerative conditions has been defined in such a way that this protein can be a target for* *therapy. It is now less of a dream that the immune system can be manipulated to protect against the TSEs. Powerful tools are now available for the management of BSE.*" In the pathogenesis of BSE, the normal process of breaking down abnormal proteins (proteolysis) does not affect the highly insoluble prions that have so far been detected in the ileum (Peyers patches), tonsils, retropharyngeal lymph nodes, spinal cord, brain and nerve ganglia, but not in muscle tissues or blood in cattle. While advances have been made in understanding the pathogenesis of BSE, the progression and timing of prion movement through the body from the point of infection to clinical disease stage will remain largely unclear, pointing to the need for additional laboratory work.

Lab research at the cellular level offers the flexibility of *in vitro* approaches emerging from the development of cell lines, with potential for new diagnostics applicable to live animals and biological materials (e.g. MBM), testing food for adulteration, and drugs for disease control (reported in *Inventory of National Research Activities in Transmissible Spongiform Encephalopathies in Europe*).

Laboratory work has also demonstrated that BSE and other TSEs can be transmitted across species lines although substantive barriers have been identified. For example, mice have been shown to be between 500 and 1,000-fold less sensitive to intracerebral inoculations of BSE than cattle. Laboratory research will also be required in investigating the mechanism of neuroinvasion, dose requirements for BSE-infection, horizontal and vertical transmission of BSE in cattle and the seemingly unlikely prospect that non-SRM (specified risk material) tissues and fluids can act as infective BSE agents. The lack of understanding regarding the pathogenesis of TSEs limits the development of improved diagnostic tests and disease control strategies.

Emerging Research Challenges in Etiology and Pathogenesis

Global

- ✓ cofactors and agents contributing to transitional forms of prion replication
- ✓ identification of pathways from exposure to CNS in cattle
- ✓ identify factors for host susceptibility and resistance
- ✓ determine the mechanism of neuroinvasion
- ✓ species barriers and susceptibility to prion transmission
- ✓ horizontal and vertical transmission of BSE in cattle
- ✓ proof of absence (of prion-based infectivity) in products, tissues and fluids such as milk, muscle, gelatin, blood and urine
- ✓ determination of pathogenesis of prion strain variation
- ✓ enhance knowledge of minimal dose of infectivity and forms of PrP associated (with infectivity)

Local

- ✓ characterize protein misfolding, prion development/expression at the cellular and molecular levels in various organs and tissues.
- ✓ collaborate with the UK laboratories in pathogenesis studies (resources, personnel, training).
- ✓ develop and utilize new transgenic animal models for the study of TSEs.
- ✓ infected animals and animal model containment facilities could be expanded.

Diagnostics and Testing

An estimated 100 companies are engaged in a race to develop a rapid, specific and sensitive test that detects pre-clinical BSE infectivity (i.e., live animal BSE test). The development of efficient, cost effective live animal diagnostics is seen as a key to both short-term disease eradication and better understanding of the pathogenesis of all TSEs. Tissues and fluids being examined for potential tests include blood, saliva, urine, and cerebrospinal fluid. Advances in diagnostic approaches beyond current postmortem tests require greater understanding and basic knowledge of prion protein structure, function, replication, and misfolding.

Enhanced knowledge applied to current technologies has yielded limited results, thus novel approaches are favored to yield major breakthroughs by increasing test sensitivity and specificity by a level of several magnitudes. Novel approaches are mainly biological, including new antibodies, enhanced PCR (Polymerase Chain Reaction), surrogate markers arising from early onset of infectivity, PMCA (protein-misfolding cyclic amplification), nucleic acid hybridization, prion amplification, microfiltration, immuno-capillary electrophoresis and cell culture assays. Enhanced imaging diagnostics capable of detecting infectivity in brain tissue would stimulate various lines of BSE-related research. Nanotechnology could provide the means for quantum advances in detection sensitivity and may lead to the development of a real-time live diagnostic test. Developments in this area require parallel advances in the basic chemistry and biology of TSEs.

Many challenges exist in the development an acceptable live animal test including the debatable evidence of markers in various tissues and blood, and the potential for variability during the lengthy incubation period. The limited availability of infective BSE material available for test verification and research signals the need for newly generated materials through transmission studies in cattle as is currently underway in the UK and Germany. Amplification of material through smaller laboratory animals and the development of bovinized transgenic laboratory mice (i.e., mice with selected bovine gene expressions) may assist in the validation of tests and further research. Intellectual property issues create restrictions on the sharing of leading-edge scientific knowledge and interfacing with relevant public research institutions.

Emerging Research Challenges in Diagnostics and Testing

Global

- ✓ development of rapid reliable highly sensitive and specific pre-clinical tests from easily accessed sources (of fluids)
- ✓ new assays capable of quantum leaps in detection; enhance existing test sensitivity (by several orders of magnitude) to detect 1 infectious unit per unit of ultimate product (e.g. 1 litre of blood, 100 grams of product)
- ✓ improved in-vitro techniques that amplify small amounts of infectious prion to enhance sensitivity of diagnostic tests
- ✓ develop enhanced imaging diagnostics capable of detecting presence of infectivity in brain tissue
- ✓ develop novel methods and reagents that detect or bind to prions; new antibodies, peptides, nucleic acids, synthetic derivatives, chimeric molecules
- ✓ identify surrogate markers or signatures for detection of prions and prion diseases
- ✓ improve techniques for propagating prions in cultured cells
- ✓ develop new *in vitro* cell systems to assay/study prions
- ✓ development of high throughput automated testing systems
- ✓ sustaining tissue banks, production and storage of infective materials

Local

- ✓ Alberta could assist with the evaluation of tests developed in the UK or other jurisdictions by providing funding, for graduate students or post-docs to work in their laboratories
- ✓ identify leading edge diagnostic tests currently under development in research laboratories globally, apply scientific due diligence and accelerate commercial development
- ✓ identify and collect positive specimens of BSE, CWD and scrapie cases from the diagnostic laboratories when available
- ✓ continuing awareness of prion research developments, including diagnostics, requires ongoing, intensive analysis such as the application of bio-informatics/knowledge management
- ✓ determine how to best use new live animal tests (when available) to meet our specific needs

Epidemiology and Surveillance

These highly applied sciences are critical in addressing the risks posed by BSE and have contributed to the focus of research studies globally. Epidemiology and surveillance requirements are unique to each country's industry characteristics, unique regionally to livestock management practices, and unique at the farm level regarding the nature of the operation (i.e., dairy or beef herd). Tracking BSE infectivity/transmissibility to and via feed and estimating effectiveness of control measures has been vital in formulating control strategies. Based on its epidemiological understanding in 1988, for example, the UK reduced the risk of BSE infectivity by 64% through the implementation of a ruminant-to-ruminant feed ban.

Effective control measures applied to animal herds are viewed as a key risk management tool. Decreased exposure to contaminated feed has significantly decreased the occurrence of BSE and is predicted by the Harvard Study to result in the eradication of BSE in the USA over a maximum 20-year period. The World Health Organization (WHO) recommends that no part of an animal affected with a TSE enter the food or feed chains. Safeguard mechanisms may be required to prevent "back door" entry of BSE materials through international trade and movement of goods.

Emerging Research Challenges in Epidemiology and Surveillance

Global

- ✓ The key R&D needs in epidemiology and surveillance are: investigation of the complete pathway of all tissues to determine their fate whether used in food, feed, medicinal products or vaccines
- ✓ examine the risks arising from slaughter methods which may contribute to contamination through exposure
- ✓ investigation of advanced data gathering techniques and enhanced survey instruments, to better define target populations for monitoring and testing
- ✓ seek evidence or proof of absence for the natural transmission of spongiform encephalopathies of animals to humans to determine the distribution of BSE infectivity in tissues and derived products from infected cattle that enter the human food or animal feed chain during the incubation period
- ✓ expand current studies on CJD infectivity of human non-CNS tissues such as those used for transplantation and in particular, blood and blood products (although there is no proven risk of CJD transmission from these sources)

Local

- ✓ Alberta has advanced expertise and capacity in epidemiology and surveillance for BSE, scrapie and CWD
- ✓ development of a DNA bar code system for DNA fingerprinting and integrating that with the National Livestock Identification System. The DNA barcode system must trace animals and meat products from farm to fork
- ✓ linkages between academics, CFIA and other government agencies provide the opportunity to develop risk mitigation measures

Control, Prevention, Therapeutics and Vaccines

While there are currently no known methods for successfully treating BSE or other TSEs, research to support control and prevention strategies is paramount to minimizing and possibly eradicating BSE in the short term. There are potential therapeutic approaches based on molecules, short chain synthesized protein molecules and antibodies that may limit the conversion of normal (PrP) to abnormal (PrP^{res}) form and effectively prevent the development of TSEs. The most promising approach appears to be rational drug design, which begins with knowledge of the tertiary structure of the protein or molecule that the therapeutic agent will target.

Vaccines and therapeutic agents are viewed with some degree of disfavour for animal populations where low to zero disease levels exist such as in Alberta. The potential for vaccines to mask symptoms rather than cure the disease could potentially compound a disease problem. Therapeutic approaches will most likely only be applied to humans.

Emerging Research Challenges in Control, Prevention, Therapeutics and Vaccines

------------------------------Global------------------------------

- ✓ develop new therapeutic agents, including antibodies, that either block the conversion of prions or disrupt the molecular mechanisms of TSE pathogenesis after conversion has taken place. The most promising approach appears to be rational drug design, which begins with knowledge of the tertiary structure of the protein or molecule that the therapeutic agent will target
- ✓ Development of efficient and effective methods of detection of prion infectivity or surrogate marker in food and feed distribution systems (i.e. rapid mass screening methods).
- ✓ Vaccines or therapeutics to prevent infection would have application in wild cervid herds where CWD is endemic or in farmed herds on highly contaminated premises.
- ✓ Develop and characterize genetic resistance in cattle and cervids.

------------------------------Local------------------------------

- ✓ Potential to do leading-edge genomic and proteomic research to develop genetic resistance in animals.

Inactivation and Decontamination

This area of research emerges in response to prion contamination risks in medical and research equipment and facilities, in the treatment of wastes from meat processing facilities, and on high-risk farms. Prions have proven to be very resistant to the usual methods of decontamination of infectious agents. Incineration, high-pressure treatment, alkaline hydrolysis and enzymatic processes have been identified as methods of eliminating or reducing infective prion material.

Emerging Research Challenges in Inactivation and Decontamination

--**Global**--

- ✓ develop safe, cost-effective disposal mechanisms for (infected or suspected) animal and rendered waste (IOM)
- ✓ identify alternative processes and products for dead animals and rendering product
- ✓ develop standard test methods for detecting contamination in environmental

--**Local**--

- ✓ develop alternative uses for all dead animal and meat processing wastes (coupled with surveillance practices), as recommended by the International Review Panel.

A Preliminary Assessment
of
Research and Development Needs and Gaps Relevant to Bovine Spongiform Encephalopathy (BSE)

An Alberta Perspective

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1.0 Introduction

A. Task at Hand

The Alberta Agricultural Research Institute (AARI), under the direction of Alberta Science and Research Authority (ASRA), and in partnership with Alberta Agriculture Food and Rural Development, determined the need to provide leadership for Alberta's efforts and investment in BSE and prion-related R&D. They identified the need to conduct a global assessment of the current state of knowledge in BSE and prion sciences and identify the key knowledge gaps that exist. Resulting from this assessment, R&D priorities for Alberta in relation to BSE and other prion diseases need to be identified. A BSE/prion science research and development strategy for Alberta will follow and be implemented with short, medium and long-term strategies and outputs. The BSE R&D priorities and Alberta's strategy will be closely linked to global efforts and the overall goal to eradicate BSE.

B. Research Questions

What role does R&D play in addressing BSE in Alberta?

R&D provides the opportunity to utilize leading-edge knowledge and technology to address critical problems. BSE is one of those critical issues and R&D has already played a key role over the past two decades in increasing our understanding of this disease (and other TSEs) and mitigating its impact on animals and humans.

In Alberta, BSE was not front and center in our research world until May 20, 2003--the day a BSE-infected cow was found in Alberta. Since then, we have begun to understand the importance of R&D in helping us unravel this mysterious disease, ensuring a continued safe food supply, minimizing human and animal health risks and ensuring the future sustainability of our livestock industry. Having a strong R&D capacity is also critical in establishing and maintaining effective relationships with our global trade partners, our beef industry and world-class scientists both nationally and internationally. It is essential that Alberta assesses its current R&D capacity and needs in relation to BSE and other prion-related diseases and then determines how it will fit into the global strategy to manage BSE and ensure its long-term eradication. "From the body of existing knowledge on prion diseases and other country's experiences emerge priorities and objectives. We then have to turn and look for partners that share objectives for collaborative research as well as countries like the UK that have research infrastructure and expertise that can be applied." (interview comment).

Why is it necessary to undertake a global assessment of the state of science and technology related to BSE?

It is essential to take a strategic approach to address those issues that are most vital to Alberta and its industry in the context of the very broad base of prion-related research that is being conducted globally. There is a wealth of knowledge about BSE and other prion-related diseases but there are also still numerous questions that remain unanswered. These unanswered questions are being worked on by scientists throughout the world but no one jurisdiction has the resources to address all the unknowns. Collaborative partnerships amongst jurisdictions are required to make long-term progress. Existing and emerging collaborative arrangements are giving focus and direction to the global research agenda. To ensure Alberta's goals are adequately addressed, it is essential that Canada and Alberta become part of these arrangements.

For the short-term, there is an immediate and vital need to develop and embrace any knowledge and scientific advance that enhances the eradication of BSE and its impact locally, nationally and internationally. Once we identify our commitments and the R&D gaps that we need to focus on, we can make strategic choices in building our scientific capacity to stay ahead of this threat on a long-term basis. If all jurisdictions are doing this on a global basis and working toward a common goal, we can reach our target and maximize the impact of our R&D investments. It will also be important that there is explicit communication regarding national research priorities and strategies and that these are incorporated into the on-going, global R&D strategies relating to BSE and prion-related diseases.

C. Methodology

A consulting team was selected to complete the first component of this task. They were asked to complete the following:

- Conduct a thorough global assessment of the current state of science [research focus, institutions and key scientists]

    - Phase one is focused on BSE
- Identify key gaps in knowledge and technology in BSE
- Identify Alberta's priorities for BSE in response to the knowledge and technology gaps identified.

The team utilized three information sources in completing the comprehensive global state of current science and technology relating to BSE (and related prion diseases):

- Expert opinion
- Existing literature
- Filed patents

Expert Opinion

While the literature on BSE is robust, it nonetheless lags behind ongoing experimentation and the expanding knowledge of researchers in the field. For this reason, over 25 key scientists were interviewed and their perspectives have been incorporated into this document. The interviews focused on the nature of existing BSE-related expertise and research facilities and also on priorities for future research. The report focuses on the following functional areas used as a template throughout the report:

- Basic Prion Science
- Diagnostics and Testing
- Etiology and Pathogenesis
- Epidemiology and Surveillance
- Prevention, Therapeutics, Vaccines and Controls
- In-activation and decontamination
- Other (research facilities, research priorities etc.)

Not surprisingly, there is both consensus and conflict regarding the nature, transmission and pathogenesis of prion diseases. A broader understanding of the consensus and conflict is helped by conversations with leading scientists and seeking their views on the most significant knowledge gaps and needs. The expert commentary can be found in Section 4 of this document.

Numerous other individuals were contacted to probe their knowledge and insights. These discussions resulted in our ability to access many additional resources that are both published and unpublished. Some of these resources are included in the accompanying Annex.

Existing Literature

The scientific literature covering BSE and BSE-related research is massive. Within this mass, there are a number of excellent documents that address the current state of research into TSEs; all are highly relevant to the more specific issue of BSE. Documents from the International Office of Epizootics (OIE), the World Health Organization (WHO), The American Institute of Medicine's Committee on Transmissible Spongiform Encephalopathies and the European Union provide excellent explanations of the current state of research and strategic pathways towards eliminating or mitigating the dangers of TSEs and potentially related diseases.

Beyond these broader review documents, scientific journals, conference presentations and miscellaneous articles provide insights in more specific areas of research. While sifting through the more than 10,000 articles that have been written on TSEs is futile, targeting of key researchers and experiments is more useful. The mass of documents available on TSEs has been honed down to 131 (Appendix A). These key documents were deemed valuable on the basis of the relevance and credibility (established through interview comments, number of publications recorded, relevance to BSE, and date of publication). The total database from which these select titles and abstracts were drawn has been developed and is available through the Alberta Agriculture, Food and Rural Development (Neil Crawford) Library for use as the initiative continues and broadens to incorporate other TSE diseases. This database was developed through existing data compiled by the TSE Secretariat (Health Canada) and through supplemental Internet searches.

Filed Patents

Filed patents provide a reasonable insight into the state of global research and the outer boundaries of knowledge that are associated with potential commercial applications. A patent search was undertaken with filings relevant to BSE and prion-related R&D (Appendix B)

D. Report Organization

Section 2 of the report provides a global review of BSE and prion-related R&D and provides a description of the global approaches, priorities and facilities. Section 3 presents a review of the science as it relates to the Alberta context and specific R&D opportunities for Alberta are outlined. Detailed comments from the 25 hours of conversation with key experts in the field follow in the primary data source section (Section 4). The final section of the report contains our secondary data sources (periodicals, scientific documents and patent searches). Approximately 130 referenced articles from some of the leading experts in the BSE and prion science R&D can be found in Appendix A. It is recommended that some of these key scientists be considered for the proposed Blue Ribbon Panel. Appendix B highlights recent patents related to BSE and prion research.

2.0 The Global Research Context

Since 1996, a number of agencies and jurisdictions—World Health Organization (WHO); the US Institute of Medicine (IOM); European Union (EU); and the United Kingdom (UK)— have evaluated, assessed, and identified research needs essential to meet the challenge posed by BSE. Many of the research needs are common to other TSEs. These numerous reviews, in full or part, are contained in annexes to this report and are the basis for the identification of the R&D needs, gaps and strategies that follow. Many of these needs, gaps and strategies are expressed in a global context as research processes across jurisdictions and science domains add incremental knowledge and new areas of science create opportunities for moving forward.

A. Globally Identified Knowledge Gaps and Needs

Globally identified R&D needs and knowledge gaps are summarized below for key functional areas in BSE R&D.

Basic Prion Science

Globally, the basic research science needs, gaps and strategies are as follows:

- clarification of the nature of the agent and identification of those structural features and their potential for variation (IOM, WHO, EU)
- determination of the function of prion protein and clarification of role where present (IOM, WHO, EU)
- determination of the mechanism of conversion to pathogenicity and replication (UK, IOM, WHO)
- pathogenesis events and mechanisms of prion dysfunction (IOM)
- extension of the molecular genetic studies of the PrP gene (and others identified as associated with TSE) in cattle (and sheep) (UK)
- identify the extent of polymorphisms in the PrP gene of humans and animals to identify genetic associations among distantly related species (UK)
- development of live animal models

Etiology and Pathogenesis

- cofactors and agents contributing to transitional forms of prion replication (IOM)
- identification of pathways from exposure to CNS in cattle (UK)
- identify factors for host susceptibility and resistance (IOM, WHO, UK)
- determine the mechanism of neuroinvasion (IOM, WHO, UK)

- species barriers and susceptibility to prion transmission (WHO)
- horizontal and vertical transmission of BSE in cattle (UK)
- proof of absence (of prion-based infectivity) in products, tissues and fluids such as milk, muscle, gelatin, blood and urine (WHO)
- determination of pathogenesis of prion strain variation (UK, IOM)
- enhance knowledge of minimal dose of infectivity and forms of PrP associated (with infectivity) (UK)

Diagnostics and Testing

- development of rapid, reliable, highly sensitive, and specific pre-clinical tests from easily accessed sources of fluids (WHO)
- new assays capable of quantum leaps in detection; enhance existing test sensitivity (by several orders of magnitude) to detect 1 infectious unit per unit of ultimate product (e.g., 1 litre of blood, 100 grams of product) (IOM)
- improved *in vitro* techniques that amplify small amounts of infectious prion to enhance sensitivity of diagnostic tests (IOM)
- develop enhanced imaging diagnostics capable of detecting presence of infectivity in brain tissue (IOM)
- develop novel methods and reagents that detect or bind to prions; new antibodies, peptides, nucleic acids, synthetic derivatives, and chimeric molecules (IOM)
- identify surrogate markers or signatures for detection of prions and prion diseases (UK)
- improve techniques for propagating prions in cultured cells (IOM)
- develop new *in vitro* cell systems to assay/study prions (IOM)
- development of high throughput automated testing systems
- sustaining tissue banks, production and storage of infective materials (UK)

Epidemiology and Surveillance

- investigation of the complete pathway of all tissues to determine their fate whether used in food, feed, medicinal products or vaccines (WHO)
- examine the risks arising from slaughter methods which may contribute to contamination through exposure (WHO)
- investigation of advanced data gathering techniques and enhanced survey instruments, to better define target populations for monitoring and testing (IOM)
- seek evidence or proof of absence for the natural transmission of spongiform encephalopathies of animals to humans to determine the distribution of BSE infectivity in

tissues and derived products from infected cattle that enter the human food or animal feed chain during the incubation period (WHO)

- expand current studies on CJD infectivity of human non-CNS tissues such as those used for transplantation and in particular, blood and blood products (although there is no proven risk of CJD transmission from these sources) (WHO)

Therapeutics, Vaccines

- develop new therapeutic agents, including antibodies, that either block the conversion of prions or disrupt the molecular mechanisms of TSE pathogenesis after conversion has taken place. The most promising approach appears to be rational drug design, which begins with knowledge of the tertiary structure of the protein or molecule that the therapeutic agent will target (IOM).

Inactivation/Decontamination

- develop safe, cost-effective disposal mechanisms for (infected or suspected) animal and rendered waste (IOM)
- identify alternative processes and products for dead animals and rendering product
- develop standard test methods for detecting contamination in environmental samples

B. Summary of Research Approaches by Selected Jurisdictions

United Kingdom:

- Strong applied scientific approach to control and move toward eradication
- Epidemiology, surveillance and post mortem diagnostics based control strategies
- Basic research program emerged in response to crisis; total R&D expenditures from 1988 to date exceed 155 million pounds
- Research priorities are:
    - Transmission; species barriers, lateral and maternal transmission, embryos and semen, effective exposure, tissue sources of infection, genetic predisposition and environmental exposure
    - Epidemiology; routes of animal product to consumption and risk of TSEs entering the food chain, targeted surveillance, origin of the epidemic, scrapie infection pathways, strain typing, modeling to understand scrapie, and scrapie as a natural disease model, clinical epidemiology and social research

- Inactivation; rendering system monitoring and measurement, biorefinement, and proteolytic enzymes
- Pathogenesis; BSE in sheep, strain typing, breed and genotype susceptibility, scrapie in sheep as prototype for infectivity, cell level studies of intestinal mucosa, infectivity in lymphoreticular and peripheral nervous systems, infectivity of edible tissues, susceptibility to infection by age and environment
- Diagnostics; live animal tests via markers, production of tissues for test development, evaluation of tests developed elsewhere, basic science of protein structure, rapid detection methods
- Basic Science; nature of infective agent, function and structural features of prions, molecular methods of strain variation, molecular and genetic basis of pathogenesis, approaches underpinning the development of markers, establishing whether prions are present in sub-clinical animals, the relationship between vCJD and BSE and prion disease susceptibility, inter-mammalian species barriers

Other EU Countries:

- Significant resources for BSE research have been developed or are being integrated into other research programs especially in Germany, France, Italy, Switzerland and The Netherlands.
- Total EU resources of an estimated 75 million Euros annually are currently being expended.
- Research priorities are:
  - Transmission; serial passage of BSE and scrapie, in sheep and mice
  - Epidemiology; established network for surveillance of ruminant TSE and standard approaches for identification of suspect cases; occurrence and stability of the BSE agent in milk and the environment, evaluation of tests to identify animals affected with BSE at pre-clinical stage
  - Diagnostics; tissue and serum tests using spectroscopy; blood test for prions, novel approaches for detection in cattle, analysis of prion proteins in tissue and blood by electrophoresis immunoassay
  - Pathogenesis; transport mechanisms from periphery to CNS; prion activity in GI tract; investigation of sporadic BSE, CNS and other BSE risk material in meat products, study of neuronal loss in brainstem nuclei of BSE cattle, role of lymphoreticular system in pathogenesis, neuropathology of animal diseases, sub-cellular relationship of infectivity, titre and prion concentration

- Therapeutics/Vaccines; synthetic peptides interfering with prion accumulations, cellular models and analysis system; prion variants and susceptibility by breed, vaccination strategies
- Basic Science; copper, oxidative stress role in synaptic transmission, development of analogous models (yeast, fungus), mouse models (transgenic) for expressing prions, in vitro models of neuronal death, development of infectable cells, characterization and chemical analysis of prions, transcription changes during infection, genetic factors related to susceptibility cell culture investigation of prion proteins, molecular determinants pf prion diseases
- Inactivation and Decontamination; evaluation of methods of detection of meat and bone meal in animal feed, CNS in food products, evaluation of heat treatment for MBM

Japan/Korea:

- Diagnostics and Detection; applied sciences for testing and evaluation of technologies; new antemortem diagnostics, NIR applications to detection
- Basic Research; transmission mechanisms of prion diseases; prion replication and potential for therapeutics, novel nanotechnology approaches to detection; development of live animal model for testing (mouse); computer modeling of prion proteins
- Epidemiology; preclinical assessment techniques for BSE

United States:

- Goals of the US program are (I0M): preventing an outbreak of BSE, preventing further spread of CWD, eradicating scrapie, reducing the incidence of CJD and developing better diagnostic and detection tools for prion diseases. Heavy emphasis on basic prion research and especially detection is evident in the US approach. While not stated, it is clear that traceability, and eradication and earliest detection are the focus.
- The infrastructure for TSE research in the US is 'small, aging' and underfunded relative to their goals. (IOM) Direction for new infrastructure is toward few large multidisciplinary facilities.
- The NIH is the largest funder spending about 0.1% of their budget for prion related research ($27 million of the total $23 billion budget). The Agriculture Research Service of USDA invested $6.6 million in applied and basic research areas. Judging by patents filed by US

researchers, considerable private capital must be attracted to this area, primarily in diagnostics and to some degree in vaccines/therapeutics.

- Few researchers (20 primary investigators) are attracted to the area; the length of time to produce results increases costs and thus the difficulty in accessing funds.
- The US Congress has appropriated funds and delegated responsibility for management of the National Prion Research Program to the Department of Defense, US Army Medical Research and Material Command (USAMRMC). They manage the resources, set strategic directions and priorities and conduct due diligence necessary to meet the goals established. They tailor funding programs to address core research undertakings and identify the best fit for basic research into novel approaches for technology development.
- For the National Prion Research Program, USAMRMC requested the Institute of Medicine to make recommendations regarding prion detection and diagnostics. A separate panel determined the investment strategy for overall prion research and will be responsible for accountability.
- Strategies employed include a range of funding approaches to attract new scientists, provide longer term funding, and enhance the basic research infrastructure.

Canada:

- Strengths are in the areas of risk analysis/assessment and applied sciences related to BSEs.
- Several strong basic research programs in neuroscience relate to prions, their structure, function and in part application of that knowledge to new diagnostics.
- Limited basic research capacity directly applicable to BSE exists; genetic and genomic programs related to resistance and a better understanding of pathogenesis are just emerging.
- A federal decision paper is currently in process. Approximately $43 million of investment is scheduled for three areas within Federal Departments (over three years).

Agriculture and Agri-Food Canada - $8 million

- A DNA Bar Code System; for DNA fingerprinting, integrating with the National Livestock Identification System
- DNA sequencing; enhancing the Bovine Genome Project
- Genetic Resistance

Health Canada - $22.8 million

- BSE/vCJD transmission and detection
- Health Protection; Screen Donors
- BSE Inactivation

CFIA - $8.7 million

- Improve speed and accuracy of prion diagnostics

        - Validate live animal tests
        - Define conditions for transmission
        - Inactivation
- The Canadian Institute of Health Research (CIHR) has been the major funder of BSE/TSE related research and has initiated a process to focus on prion disease related research, and as a sponsor of prion disease workshop, has identified priority research themes (focused on BSE and other TSEs):
    - applied science endeavors in pathogenesis, identification of risk materials, tissue distribution, science based risk assessment, species barriers, host range, environmental protection, and dose response
    - improved laboratory models; antemortem diagnostic and screening tests
    - characterization of infectivity, prevention and therapeutics
    - decontamination in various sites (hospitals, farms)

  A supplemental informal session to identify specific research focus and strategies was undertaken and material from this session may become available at a future date.
- Several Canadian researchers are partners in international research programs, in basic research related to prion structure and blood based diagnostics.

C. Key Facilities and Locations

The following table provides a global overview of key R&D facilities involved in BSE and Prion-related diseases:

Prion Research Locations

Canada	University of Toronto Centre for Research into Neurogenesis	Basic Science Diagnostics
	Canadian Food Inspection Agency Winnipeg and Lethbridge	Diagnostic; Pathogenesis Level 3 and 4 facilities
	Health Canada Ottawa, Winnipeg	Basic; Diagnostics- blood safety (CJD)
	Western College of Veterinary Medicine Saskatoon	Epidemiology (beginning)
	Alberta Agriculture, Food and Rural Development Edmonton	Diagnostics
	University of Alberta Edmonton	Basic Science (Genomics, Proteomics)

United States	National Animal Disease Center, USDA Ames, Iowa	Diagnostic; Epidemiology
	NIH Rocky Mountain Laboratory Hamilton, Montana	Basic Prion Science
	Department of Neurology, Biochemistry and Biophysics; University of California San Francisco	Basic Science; Diagnostics
	Veterinary College, Colorado State University Fort Collins, Colorado	CWD pathogenesis
	Department of Veterinary Sciences, University of Wyoming Laramie, Wyoming	CWD pathogenesis, Diagnostics
	Division of Neuropathology, University of Maryland School of Medicine Baltimore, Maryland	Basic Science (etiology)
	NIH, Bethesda Baltimore, Maryland	Pathogenesis (blood and food safety)
	Institute of Neuropathology, Case Western Reserve University Cleveland, Ohio	CJD
	Veterans Affairs Medical Center, Medical Research Center Baltimore, Maryland	Basic Science
	The Salk Institute for Biological Studies San Diego, California	Basic Science, Vaccine
United Kingdom	Veterinary Laboratory Agency, DEFRA Weybridge, England, UK	Pathogenesis, Diagnosistics, Epidemiology
	Roslin Institute Roslin, Midlothian, Scotland	Diagnosistics, Pathogenesis, Epidemiology
	Biomedical Sciences, University of Edinburgh Edinburgh, Scotland	Basic Science, Inactivation
	University College Dublin, Ireland, UK	Basic
	Centre for Epidemiology of Infectious Diseases, Department of Zoology, University of Oxford Oxford, England, UK	Epidemiology
Continental Europe	National Reference Center for Human Prion Diseases, Institute of Neuropathology, University Hospital of Zurich Zurich, Switzerland	Basic Science
	Robert Koch Institute Berlin, Germany	Basic Science, Diagnosis

Japan	Neurological Institute, Faculty of Medicine, Kyushu University Fukuoka, Japan	CJD
	Prion Disease Research Centre, National Institute of Animal Health Tokyo, Japan	Basic Science
	Nippon Institute of Biological Science Tokyo, Japan	BSE
	National Institute of Neuroscience Tokyo, Japan	Basic Science
	Kobe University, Faculty of Agriculture	Diagnostics
	School of Agriculture and Life Sciences, University of Tokyo Tokyo, Japan	Basic science and Diagnostics

3.0 Knowns, Challenges & Opportunities - Alberta Perspective

A. Science and Technical Review

Using the global context as the starting point, this section focused the knowns, challenges and opportunities for Alberta in relation to BSE R&D.. The knowns, challenges and opportunities are organized into the key focus areas.

Basic Prion Science

Research efforts, over the long term, to enhance knowledge of prions hold the most promise for understanding TSEs. Basic research in prion science, dating back to the early 1980s and the postulation of prions as an infective agent, gained significant momentum with human cases of vCJD in the UK in the later 1990s and the subsequent linkages between vCJD and BSE. Preliminary studies have shown that the immune response is absent, the pathways from ingestion to infectivity may or may not be affected by numerous factors, there are lengthy periods between exposure and clinical evidence of disease, and that host susceptibility is highly variable. The latter point has encouraged the integration of genetic and biotechnology based initiatives for pathogenesis and resistance studies. Linkages across animal and human health areas of studies are vital to enhancing knowledge at the basic research level.

Impediments to basic prion research include the scarcity of laboratories meeting bio-hazard and bio-terrorism criteria; limited access and supplies of infected tissues; the high cost of large animal studies; significant infrastructure requirements; risks relative to decontamination and the lengthy period to yield results. Furthermore, all such experiments are subject to CFIA approval in Canada.

While there may never be consensus with respect to the origins of BSE, debate continues as research advances fill in the knowledge gaps related to structure, replication and indeed, the role of normal prion proteins, which in recent studies have been detected in memory function as well as in certain bacterial infections.

Prion strain variation has been identified for most TSEs while BSE is a notable exception to date. Recent studies in Italy, Japan and France, however, have created unease with the concept of a single strain being the global infective agent. This area merits continued study to ensure knowledge of variation in strains, should it occur in the future, exists and whether pathogenesis mechanisms are impacted.

Knowledge and Capacity

- The majority of scientists accept that prions (i.e., misfolded prion protein or PrP^{sc}) are essential to the expression or development of TSEs.
- There is wide recognition that other factors (i.e., catalysts, other infectious agents, mineral status, genetic predisposition, environment, etc.) may be necessary for TSEs to occur.
- The immune system does not recognize prion infection is occurring.
- There are over 20 known strains of prions in scrapie.
- Knowledge of the normal functions of prion proteins and abnormal folding of proteins is beginning to emerge.
- TSE models exist in various species (mice, hamsters, primates, sheep, goats).
- TSEs are characterized by long incubation periods, linked to genetic factors and cross-species barriers.

Unknowns & Challenges

- Lack of knowledge or understanding of normal prion protein and prions is the main obstacle to developing more sensitive and specific diagnostics.
- Incomplete understanding of the structures of prions, molecular structures and mechanisms of replication, misfolding phenomenon, and TSE pathogenesis.
- One strain of BSE has been identified, but whether others exist or are evolving is currently unknown.
- Natural genetic resistance to BSE has not been observed in cattle, but resistance has been identified in sheep, elk and humans to other TSEs.
- Incomplete understanding of species barriers (e.g., sheep to cattle / cattle to cervids).
- Variation in response to proteinase K enzymes (occurs).

- Animal breed differences in susceptibility to TSEs (may occur).

Research Priorities for Moving Forward

- Understand the structures of prions, molecular structures and mechanisms of replication and the misfolding phenomenon.
- Better understand normal (PrPc) as well as abnormal (PrPsc) proteins.

Alberta Opportunities

- Strengths in proteomics, genomics, micro-fluidics and bio-informatics, potentially applicable to advancing the knowledge of BSE and other TSEs.
- Potential to build partnership with The Synchrotron Institute in Saskatoon to better understand protein structure and folding.
- The Level 3 laboratory facility in Lethbridge (CFIA lab) appears to be under-utilized.
- AAFRD and University of Alberta have proposed the construction of Level 3 facilities.
- Diagnostic, research and regulatory functions critical to respond to the directions from the International Expert Panels on BSE (recent Canada/US panel included) are available in Alberta.
- Alberta has recognized diagnostic and testing capabilities for BSE and CWD.
- Alberta has recognized research expertise in epidemiology, diagnostics and testing methodologies that can and does collaborate with researchers and scientists in other locations in Canada, the UK, Japan and the US.

Etiology and Pathogenesis

Research at the basic and applied levels is vital for both eradicating BSE and ensuring safety of food and feed products. Tracing infectivity from exposure through its transition to a disease of the CNS brings together all the components of the science of TSEs and contributes to the refining and emergence of enhanced and new diagnostic tools. Species transmissibility (BSE in sheep), maternal transmission and ensuring absence of infectivity through blood and muscle tissue are preventive research strategies relevant to BSE: "Pathogenicity and virulence of BSE is consistent and BSE has limited tissue involvement outside the CNS" (*TSEs in Animal*

Populations – Fact and Fiction, Conference Proceedings; September 10-11, 2003; Fort Worth, Colorado).

A large (300 head of cattle) pathogenesis study is underway in the UK. The prion theory is accepted as an important component, but there are possibilities that other confounding factors are involved in the cause of TSE diseases. These include the role of metal cations, RNA viruses, spiroplasma, and organophosphates. The actual etiology of TSEs remains unclear.

Studies at the cellular level offer the flexibility of *in vitro* approaches emerging from the development of cell lines, with potential for new diagnostics applicable to live animals, inactivated materials (MBM), testing food for adulteration, and drugs for disease control (reported in *Inventory of National Research Activities in Transmissible Spongiform Encephalopathies in Europe*).

Knowledge and Capacity

- Limited research has been done on the pathogenesis of BSE (and CWD). Prions have been detected in the ileum (Peyers patches), tonsils, retropharyngeal lymph nodes, spinal cord, brain and nerve ganglia. It has not yet been found or identified in muscle tissues or blood in cattle.
- The aggregation of prions in the CNS is used as a diagnostic benchmark for TSEs.
- There are documented cases of genetic susceptibility/resistance in animals, animal models (scrapie, CWD) and humans (vCJD).
- There are reports of prions occurring in muscle tissue of animal models (mice, hamster) and human cases of CJD.

Unknowns & Challenges

- Progression and timing of prion movement through the body from the point of infection to clinical disease stage is largely unclear.
- Greater understanding of the factors involved in the etiology of BSE in required.
- The lack of understanding of the pathogenesis of prion diseases limits the development of improved diagnostic tests and disease control strategies.

- The routes of transmission and host susceptibility of TSEs lack full understanding.
- Better definition of the species barriers to understand risks and disease transmission possibilities (e.g. BSE infection in sheep).
- Require increased knowledge regarding infective dose requirements via feed for BSE in cattle.
- Verify the absence of prions in blood and muscle tissue of cattle.

Research Priorities for Moving Forward

- Improve the characterization and understanding of the pathogenesis of BSE in cattle by conducting experiments with adequate numbers of animals.
- Development of improved animal models (e.g. transgenic mice and others) to enhance understanding of the pathogenesis of BSE and TSEs.

Alberta Opportunities

- Characterize protein misfolding, prion development/expression at the cellular and molecular levels in various organs and tissues.
- Collaborate with the UK laboratories in pathogenesis studies (resources, personnel, training).
- Develop and utilize new transgenic animal models for the study of TSEs.
- Infected animals and animal model containment facilities could be expanded.

Diagnostics and Testing

The development of efficient, cost effective antemortem diagnostics is seen as a key to both short-term disease eradication and better understanding of the pathogenesis of all TSEs. Diagnostics is a priority research strategy in all global jurisdictions that are challenged by TSEs and public and private sectors have committed significant resources to this effort. Private laboratories have identified significant potential opportunity for diagnostic tests in surveillance, eradication, traceback and assurance programs as a vital component of global market access.

Advances in diagnostic approaches beyond current postmortem tests require greater understanding and basic knowledge of prion structure, function, replication, and misfolding. Enhanced knowledge applied to current technologies has yielded limited results. Novel approaches are, however, favored to yield major breakthroughs by increased test sensitivity and specificity by a level of several magnitudes. The novel attempts are mainly biological, including new antibodies, enhanced PCR (Polymerase Chain Reaction), markers arising from early onset of infectivity, PMCA (protein-misfolding cyclic amplification), nucleic acid hybridization, prion amplification, immuno-capillary electrophoresis and cell culture assays. The challenge of developing live animal blood tests rests with the debatable evidence of markers in blood and the potential for variability during the lengthy period of disease onset subsequent to ingestion of infective material.

The most desirable outcome is a live animal test that could be administered efficiently and economically and would serve as a key tool for eradication of the disease and ongoing surveillance of large numbers of animals. Physical sciences combined with chemical and biological science is considered to hold promise and merits attention for the creation of this test. Nanotechnology could yield increased sensitivity by a quantum advance, requiring advanced knowledge of associated chemical and biological factors resulting in the ultimate of a real-time live diagnostic test. France has taken a lead role in creating a live animal test and the US is also investing approximately $40 million for test development. The potential of microfluidics with other combinations of science endeavors available within Alberta merits further evaluation.

Knowledge and Capacity

- Over 100 institutions and companies are involved in the race toward development of an antemortem test (post mortem testing is already in place).
    - Dr. Neil Cashman (University of Toronto) has 3 candidate tests under development for TSEs (funding from CIHR).
    - MDC, Inc. in Calgary and GeneThera in Colorado claim to have blood tests, but neither have not been published or evaluated.
- Tissues and fluids being examined for potential tests include: blood, saliva, urine, and cerebral spinal fluid.

- Novel diagnostic approaches include items such as microfiltration, surrogate markers, and PCR techniques.
- Intellectual property issues create restrictions on sharing of leading-edge scientific knowledge and interfacing with relevant public research institutions.
- CFIA is responsible for approving diagnostic tests and access to the infectious material required to validate tests.

Unknowns & Challenges

- A roadblock to evaluating live animal tests is the lack of available infected tissues.
- Tests that are sensitive, specific and able to detect disease in early stages, i.e., detecting infection at pre-clinical stage, are required.
- Maintain and develop capacity in contributing domains and technologies, such as mass spectrometry, genetics (marker technology, engineering), blood testing, molecular detection, and proteomics.
- Development of improved validation approaches through live animal models (e.g. transgenic bovinized mice).

Research Priorities for Moving Forward

- Rapid validation of potentially useful live animal tests.
- Increased availability of known materials (tissues and fluids) to facilitate the on-going validation of tests.

Alberta Opportunities

- Alberta could assist with the evaluation of tests developed in the UK or other jurisdictions by providing funding, for graduate students or post-docs to work in their laboratories.
- Identify leading edge diagnostic tests currently under development in research laboratories globally, apply scientific due diligence and accelerate commercial development.
- Identify and collect positive specimens of BSE, CWD and scrapie cases from the diagnostic laboratories when available.

- Continuing awareness of prion research developments, including diagnostics, requires ongoing, intensive analysis such as the application of bio-informatics/knowledge management.
- Determine how to best use new live animal tests (when available) to meet our specific needs.

Epidemiology and Surveillance

These highly applied sciences have been critical to address BSE and have contributed to the focus of research studies globally. Tracking BSE infectivity/transmissibility to and via feed and estimating effectiveness of control measures have been vital to the formulation of control strategies. Debate of the origin of BSE is divided among those who view evidence supporting a single source (eg., scrapie in sheep or spontaneous mutation in cattle) versus multiple sources (environmental, toxic materials). Epidemiology and surveillance requirements are unique to each country's industry characteristics, regionally to livestock management practices, and at the farm level whether dairy or beef operations.

Knowledge and Capacity

- Feed source infectivity is identified as the key route of infection for BSE.
- Downers, disabled and cattle expressing neurological signs have a higher risk of BSE infection.
- Cattle that have been fed MBM early in their life and were born before the feed ban pose the highest risk for BSE.
- The majority of beef cattle are processed for meat at a young age (less than the mid-point of the incubation period).
- Alberta beef cattle production methods (raised on range) are unlikely to expose cattle to infected feeds, particularly after the 1997 feed ban.
- CWD is known to transmit horizontally in cervids which raises concerns about inter-species transmission of CWD.
- All elk and deer in Alberta that die or are processed for meat, are tested for the CWD in order to eradicate the disease.

- The potential for BSE cases in Alberta is related to the imposition of the 1997 feed ban (i.e. opportunity for recycling of infected feed prior to the ban). The imported BSE positive animal in 1993 raises the possibility that there were other imported infected animals that entered the feed system.

Unknowns & Challenges

- Infectivity and prevalence of BSE and CWD in animal populations are not well understood due to the lack of adequate surveillance and diagnostics.
- The lack of live animal diagnostic tests limits the ability to determine the pattern of disease in animals.
- Mass application of postmortem screening of slaughtered animals is costly.

Research Priorities for Moving Forward

- Meeting the surveillance requirements as recommended by the International Panel [Reference: *Report on Measures Relating to Bovine Spongiform Encephalopathy* (BSE)] in the United States and Canada.
- Conduct risk assessment studies to formulate mitigation measures and disease management strategies (i.e., linking risk assessment data with research studies).
- Adequate levels of surveillance to understand disease transmission in animal and human populations.
- Develop a system to sample and test dead animals on farms for TSE surveillance.
- Increased knowledge of the TSE disease process and factors influencing its expression through epidemiological study.
- Continue monitoring geographic distribution of CWD in wild cervid populations and possible links to occurrence of BSE.

Alberta Opportunities

- Alberta has advanced expertise and capacity in epidemiology and surveillance for BSE, scrapie and CWD.

- Development of a DNA bar code system for DNA fingerprinting and integrating that with the National Livestock Identification System. The DNA barcode system must trace animals and meat products from farm to fork.
- Linkages between academics, CFIA and other government agencies provide the opportunity to develop risk mitigation measures.

Control, Prevention, Therapeutics, Vaccines

Research to support control and prevention strategies is paramount to minimize and eradicate BSE in the short term. There are potential therapeutic approaches based on molecules, antibodies and short chain synthesized protein molecules that may limit the conversion of normal (PrP) to abnormal (PrP^{res} or ^{bse}) form to provide efficacy in the treatment of TSEs.

Basic scientific investigation has only begun to identify the constructs of prions that may detect vulnerability to preventive therapeutics, applicable to both humans and animals. Vaccines and therapeutic agents are viewed with some degree of disfavor for animal populations where low to zero disease levels exist such as in Alberta.

Knowledge and Capacity

- There are currently no known methods for successfully treating TSE diseases.
- Decreased exposure to contaminated feed has significantly decreased the occurrence of BSE and is predicted by the Harvard Study to result in the eradication of BSE in the USA over a maximum 20 year period.
- The emergence of therapeutic agents and vaccines is highly dependent upon advances in basic science and understanding of TSE diseases.
- Therapeutic approaches will likely only be used for humans.
- Eradication or effective management of BSE in cattle populations will reduce/eliminate its impact. Vaccination may interfere with diagnostic tests and recognition of exposure.
- Some evidence exists that the development of genetically resistant animals is possible (accomplished in mice and elk). Preliminary work in Korea indicates that there are possibilities for transgenic developments in cattle.

Unknowns & Challenges

- Eliminate exposure of animals and/or people to infectious agents of TSEs.
- Meet the WHO recommendation that no part of an animal affected with a TSE enters the food or feed chain.
- Safeguard against the "back door" entry of BSE material as a result of international trade or movement of goods.
- There currently is no vaccine available for either animals or humans.
- Human medical studies for the prevention and treatment of CJD may offer hope for advances for CWD and BSE control.

Research Priorities for Moving Forward

- Development of efficient and effective methods of detection of prion infectivity or surrogate marker in food and feed distribution systems (i.e. rapid mass screening methods).
- Vaccines or therapeutics to prevent infection would have application in wild cervid herds where CWD is endemic or in farmed herds on highly contaminated premises.
- Develop and characterize genetic resistance in cattle and cervids.

Alberta Opportunities

- Potential to do leading-edge genomic and proteomic research to develop genetic resistance in animals.

Inactivation and Decontamination

This area of research emerges in response to several factors. Safety and prevention of transmission in potentially infective environments including testing facilities, veterinary and medical facilities, treatment of wastes from facilities, and potentially contaminated animals and products is essential. Temperature, pressure and enzymatic processes including treatment vessels are under active research in most jurisdictions. Issues of environmental contamination have been identified, though basic knowledge of infectivity from incubation or persistence in the soil or the environment is limited.

Knowledge and Capacity

- CFIA (and EU and USDA) have established standards for decontamination of equipment and laboratory facilities.
- Prions are resistant to destruction but can be inactivated with alkaline hydrolysis and high temperature incineration.
- Very high pressure applications have been shown to reduce the number of prions in biological material.
- It has been shown that prions can survive in the environment in infected materials.
- The rendering process currently used in Canada reduces the level of infectivity by a factor of 10.
- Recent publications indicate that enzymes (keratinase) may destroy prions.

Unknowns & Challenges

- Determine the duration and conditions under which prions will persist in the environment
- Removing infectivity from MBM and/or animal products.
- Develop effective means to decontaminate or eliminate the prions from the environment in highly contaminated CWD (and scrapie) premises.
- Adequate and effective sterilization of medical equipment and instruments is required.

Research Priorities for Moving Forward

- Develop methods to treat or inactivate prions in MBM and/or SRM to prevent transmission.
- Investigate enzymatic treatment of facilities, instruments, and ultimately apply to waste management of MBM, carcasses and farm environment (e.g., composting).
- Remove CJD (or vCJD) infectivity from blood or blood products used for transfusions or treatments in humans.
- Environmental decontamination for CWD.

Alberta Opportunities

- Develop alternative uses for all dead animal and meat processing wastes (coupled with surveillance practices), as recommended by the International Review Panel.

B. Summary and Implications for Alberta – Moving Forward

Alberta has considerable R&D strengths that are recognized both nationally and globally. Alberta's key areas of strength are in the following areas

- epidemiology, surveillance and diagnostics as applied to BSE and CWD
- advanced proteomics, bioinformatics, biochemistry and bovine genomics at Alberta's three universities
- potential applicability of basic research skills and capacity in health sciences and emerging capacity in nanotechnology
- established collaboration nationally and internationally (UK, EU, US and Japan)

Building on these strengths and understanding the key R&D priorities in BSE and prion-related science, the R&D priorities that are most applicable to Alberta are the following::

- basic research in protein folding mechanisms
- genetic resistance of cattle
- enhanced capacity to study pathogenesis linked to current epidemiology, diagnostics and surveillance

It is recommended that Alberta's expertise across all jurisdictions, related to animal sciences and animal health, should be thoroughly reviewed with respect to the emerging challenges facing the livestock industry. The total value, contribution and sustainability of Alberta's livestock industry requires advances on a number of fronts that can, in part, be addressed through significant science and technology investments. The public resources located at Lethbridge (AAFC, ADRI), Lacombe (AAFC), Edmonton (UofA, AAFRD), Calgary (UofC), Vegreville (ARC) and linkage to WCVM and VIDO in Saskatoon represent a sound resource base. The potential of enhanced capacity in genomics/proteomics and novel diagnostics would help create a critical mass capable of attracting leading edge scientists and scientific endeavor and global animal health commercial interests. Modest incremental investment in selected research areas combined with leadership providing strategic focus and direction could complement Alberta's future direction in life sciences.

It is noted that basic science endeavors requiring long-term commitment should be assessed for their broader application, especially to human diseases arising from related origins (of disease).

While development of therapeutics and vaccines may represent a control strategy for BSE and a commercial opportunity, direction should be established which considers the limited exposure to BSE and capacity to eradicate BSE in Alberta and Canada.

Future progress toward the eradication of BSE in Alberta and Canada will require globally recognized science capacity in selected areas of science, collaboration, and investment of both the public and private sector funds within and outside Alberta and Canada to address the research gaps most crucial to Alberta. A national strategy for BSE research would help ensure effective use of limited resources and provide focus on the priority research gaps most critical to Canada and Alberta.

4.0 Primary Information: Expert Commentary

Approximately 25 key scientists in the field of BSE and prion-related research were interviewed to gain further insight into the leading-edge R&D that is being conducted globally. The names of the individuals will be held confidential but the list included representatives from each of the following organizations:

- Agriculture and Agri-Food Canada, Population and Public Health Branch
- Alberta Agriculture, Food and Rural Development
- Veterinary Laboratories Agency, Department for Environment, Food and Rural Affairs, UK
- Animal Disease Surveillance, Canadian Food Inspection Agency, CFIA
- Animal Laboratories Services, CFIA
- Biochemistry Department, University of Alberta
- Bureau of Microbial Hazards, Health Canada
- Canadian Institutes of Health Research, Institute of Infection & Immunity
- Centre for Infectious Disease Prevention and Control, Health Canada
- Department Biochemistry & Molecular Biology, University of Calgary
- Department of Agricultural, Food and Nutritional Science, University of Alberta
- Department of Medicine, University of Toronto
- Department of Veterinary Sciences, University of Wyoming
- Departments of Medicine and Biochemistry & Molecular Biology, University of Calgary
- Faculty of Medicine and Dentistry, University of Alberta
- Foreign Animal Diseases, CFIA
- Genome Prairie/Genome Canada
- National Laboratory for Host Genetics and Prion Diseases, National Microbiology Laboratory, Health Canada
- Private Consultant (health, biotech and investment)
- TSE Secretariat, Health Canada
- United States Public Health Service
- Western College of Veterinary Medicine, University of Saskatchewan
- Western Laboratories Network, CFIA
- Vaccines and Infectious Diseases Organization

Their comments and insights are documented in the following pages and are categorized into the six focus areas used throughout this report. The comments provide interesting perspectives on the numerous issues involved in BSE and BSE-related R&D.

Please note that we also contacted numerous other individuals to probe their knowledge and insight which resulted in our ability to access many additional resources that are both published and unpublished. Some of these resources are included in the accompanying Annex.

Basic Prion Research

- Regarding genetic variation, there have been three other chromosomal regions in cattle implicated in susceptibility to BSE. These are apart from the prion gene and we would propose that we look at genetic variation across those three other regions in cattle populations. Then use that information in British populations of animals to see if we get any better association with susceptibility to prion disease. The whole reasoning behind this is that Britain is the only region with a good population resource to work on BSE as well as first hand experience dealing with the disease.

- We also want to look at variation across the prion region of the genome because to date there has not been any susceptible genotypes identified for BSE as there have been for scrapie. With scrapie and humans there are genotypes which are susceptible to prion disease. In cattle there has been no evidence for that within the prion region yet. The prion region in the gene has been sequenced at the Clay Centre of the USDA and others.

- The tough thing about prions is they have multiple other functions. With the genome being clarified…there are proteins that are very closely related to prions that have very different functions. Can these become infectious is the key question. Finding that prions may be key component to memory -coming back to Alzheimers.

- There are 11 known strains of prion diseases in humans, 21 in sheep and so far, only 1 in cattle. Japan has noted one abnormal prion case for under 30 mos. cattle. Italy has 2 older cattle cases of interest and France has 3 cases of interest (uncertain strain). The question is whether we are on the cusp of another strain and whether we need more or new control measures.

- The genetics elements of the disease… 50% say there is DNA involved (that there is a virus involved in this), another 50% say no. If you use the titrate (measuring volume required to carry out a reaction) experiments that we used in blood I believe there is a virus involved. The way it titrates out is as a virus… a protein and a virus. It doesn't titrate out as a pure protein. We haven't found any DNA associated with it, it is still a protein hypothesis, but I think the catalyst might be a virus that produces a protein to bring down the temperature, or something in the monocyte because this protein, when it enters any human or animal gets eaten up right away by the monocyte in the GI area. At the monocyte level, there must be an interaction with that protein to change its structure.

- Dr. Stephen Moore is part of the international bovine sequencing project and the Canadian sequencing - bovine sequencing project is important in this regard. This will give us the basic genetic and biological information that will allow us to distinguish one cow from another, susceptibility to one disease or another, perhaps including BSE and that may tie into a lot of these efforts. Also looking down the road, can we agree that there are cows that are resistant to this disorder? This might arise from development in genetic technology. Korea has developed work in this area. They have identified variant genes that are naturally occurring in other animals that don't get this disorder. So you can switch genes from one animal to another, and it looks like it could be possible to do this in cows. There is consumer hesitation in working with genetically modified organisms, but if we could generate strains of cows resistant to BSE, that might be the first time GMO are seen to do something good. We should undertake some catalytic investments, so that if this becomes a promising approach, we are there at the front of the tide.

- The test may have to pick up subtle variations in the infected prions. This is technically and scientifically a big challenge to know exactly what you've got, and its precise correlation with either bovine disease or in particular human disease. The connection with memory is of interest and may bring forth ideas of how this is spread. This suggests an additional symptom (circadian…sleep/wake…rhythms also). Prions may be related to multiple functions, including motor functions which are related to the cerebellum.

- The question as to whether there is another participating molecular species is completely up in the air. Even Prusiner contends that there is probably something else involved. There are other people that think that the other thing that is involved might be anything from a host protein to DNA to an undetected retrovirus. Basic science is still at a point where we cannot say that we have identified the infectious agent.

- The concept of a misfolded protein acting as a template that constrains other proteins to adapt to its form still is not backed by a fine explanation. The whole question of intermediate species between the normal and fully misfolded protein is a fascinating field that will probably yield fruit over the next two years. Jiri Safar and Stan Prusiner's group (UC San Francisco) have already done some interesting work in this area (high pressure used as a tool). In France, researchers have already shown that there are a species of intermediates that are partially denatured that are different than intermediates that can be identified after heating. This is work that is being done, in part, by Reinhard Lange at French Institute of Health and Medical Research (INSERM) in Montpellier, France.

- There is a need to link experts in the structural functions of proteins to the experts in biology. This is not happening… either people are working on structure or they are working on biology.

- Although it has been validated with a Nobel Prize in 1997, there are so many missing pieces in our understanding of how protein form A gets to protein form B. It's almost as if it still hypothetical. The actual biochemistry of this conversion needs to be elucidated. There are people doing work in how conversion occurs: Byron Caughey in Montana and John Collinge (Imperial College, London) and Neil Cashman (University of Toronto) who is also working on the basic pathogenesis of how protein conversion works.

- A lot of people have found different functions for the normal cellular isoform of the prion protein but nobody knows for sure. There is a wonderful paper in press in the Journal of Science where somebody has taken neurons and cross-linked cell surfaced prion protein using antibodies and it triggers a cell death signal. That makes some sense, because as abnormal prion protein accumulates in the extracellular space, it is probably doing the same as the antibodies...cross-linking normal cellular isoforms at the cellular surface and causing cells, causing neurons to die. Why would you have an apoptosis receptor at the cell surface…I don't know, but it is interesting.

- On the issue of prion proteins being related to memory…that was not the prion protein itself, but a protein involved in memory in an organism. A scientist noted that the protein changes conformation when a memory is being established in a marine mollusk… it not only changes conformation, it propagates that changing conformation when it comes in to contact with newly transcribed and translated proteins. So it is a prion concept working in a different molecule. This process might ultimately tell you how these proteins can influence others to change.

- Dr. Stan Prusiner went through and synthesized the prion protein completely from amino acids and then with his synthetic protein then produced the disease and that was the final nail in the coffin to the fact that there is no nuclei acid associated with the transmission of BSE. He knocked out the prion gene in the mouse and then showed you couldn't produce the disease, so he proved that the animal couldn't get the disease. Then he put the gene back in and it became diseased.

- We've always assumed once you know the amino acid sequence of a protein, you would know how that protein would fold. What has surprised us since 1993 is that the amino acid sequence doesn't always determine how a protein will fold, so there has begun a whole new science around protein folding. It is surprising how many diseases are related to protein folding. Rather than getting stuck on a single disease, the priority is to say how can we address and build up our science to address issues around protein folding in its relationship to disease in animals and humans. I think it will be a big issue in a number of human diseases over the next 10 to 15 years. So you build up something that can be applied with these diseases.

- The leaders in all this will studying the nature of protein folding. The leaders will still be protein bio-chemists and molecular biologists that are studying the nature of protein folding.

- The protein question. This is a very difficult question to discuss because the vast majority of the field has either converted to a belief that it is only a protein and we don't need to look further, or they believe it is not worth worrying about, because regardless of whether it's a protein or there is some nucleic acid attached, you have the undisputed central role in pathology and in diagnostics of the PrP protein. On the other hand there is a small, rather intense band people that refuse to think that it is just a protein and think that it is a very important question. According to Dr. Prusiner, the attempt to purify it and then reintroduce it into a susceptible host has been achieved a long time ago. My thinking is that this hasn't yet been accomplished because of the devilishly difficult task of purifying this thing which has this all you ability of Teflon, and spreads through all density gradients and so on. So no one has purified this to homogeneity in my thinking.

- The underlying concept for Byron Caughey's work, the line of work where he has this *in vitro* conversion system so highly refined that really all he needs is highly purified PrP fibrils and a recombinant tracer that is able to be converted. That line of work is very, very interesting because if it serves as a kind of *in vitro* surrogate for the nature and the intensity of the barrier to infectivity between species it could potentially teach us a lot about where the risks are for interspecies transmission. I don't actually think that species barriers are that simply defined by the binary interactions between the mis-folded and the not yet mis-folded PrP of the host. I think there is probably more to it.

- It is very important to understand the structural set up of mis-folded PrP aggregates. And that so far has not been possible because of the extremely poor solubility of those misfolded prions.

- Amplification through mice…good for basic research, but if you want to do applied work, I think you need to study it in the host.

- In Canada only two people are working on the basic mechanism of protein folding: Dr Cashman (University of Toronto) and Andrea LeBlanc (McGill University).

- Because of the dominance of Prusiner's ideas which are classically reductionist, they basically say, this whole sphere of phenomena can be reduced to the behavior of the single proteins, and that to you draw your boundaries for scientific endeavor. Because of the dominance of that thinking, prion science is probably a decade behind the rest of applied biology. It is important to begin applying new biology, by which I mean the sort of system-level approach that is exemplified in genomics and proteomics and Bio Informatics…to the define the problems, not just solving them but framing them in a systems approach. What you can find in the genetics domain just isn't there in prion research. This is about the way of squeezing new information out of the system by applying these broad-based approaches to prion biology.

Etiology and Pathogenesis

- The Canadian part of the bovine genome sequencing project is mainly carried out at the genome sciences Center in Vancouver where we are sequencing full-length cDNA (gene transcripts) from cattle. Genome Canada is putting $5 million towards that. We said that the tissues we should concentrate on are those that are implicated in the transmission and development of the prion disease. And we've collected a number of those tissues already and we are making some cDNA libraries to go into their sequencing pipeline. It has to be said that the Genome Canada project is not a science project (they're going to sequence 10,000 full length cDNAs for the genome sequencing effort; nothing special other than produce a sequence).

- If we understand more about how the disease develops, we may end up with a better way of testing animals earlier. Looking at what tissues are involved (this is what cDNA research can help); we might be able to test white cells at a certain age to catch things before they progress further, it may be a way of eliminating the disease. Understanding of how disease progresses from infection to symptoms is key. We are trying to collaborate in this endeavor as we combine skill sets.

- On BSE, there's not good evidence of a genetic predisposition. For humans, there is a huge push towards that. What is the susceptibility of our population? That whole link of variant CJD… there are new strains of BSE that look a lot more like variant CJD in their pathology than the generic or mean strain of BSE. That work is out of Italy. We're only into the first chapter on prion diseases. As tests and levels of sensitivity improve, then we will find a whole bunch more prion diseases out there that we have not yet identified.

- Do you assume that only a few had a significant dose or that there is genetic resistance…maybe it is an age thing. UK is looking at genetic resistance (don't know where it is at) across animals and breeds. There is commercial interest in genetically resistant cattle.

- The gene encodes a normal protein and then an abnormal protein after proteolysis, so what happens after partial proteolytic cleavage and abnormal refolding of the protein? All mammals contain homologous genes encoding this protein. All mammals have it, however not all mammals are susceptible to this disease. In some mammals the gene encodes a protein that is very susceptible to this abnormal folding. In some animals the protein is more robust and it tends not to refold into the abnormal prion protein. So the answer to what is going on that is different from one animal to the next or even one strain to the next depending on the precise coding sequence. And maybe also it depends on the abundance of this strange hypothesized autocatalytic RNA that might mediate the transformation to the abnormal structure. Once you get it to the abnormal structure, it appears to trigger, like lego blocks with an odd shape… it changes the other blocks into the odd shape and like a chain reaction that results in more abnormal folding, resistant to further proteolosis that should be cleaning up abnormal proteins and degrading them, somehow become resistant to it, you get these abnormal plaques that form in the brain and sometimes other tissues in the body… that leads to very strange neural physiology, and also triggers programmed cell death or apoptosis… and this seems to go faster. Animals can go from normal state to death in as short a time as six months.

- Alternative theories for origin of BSE is that the immune system screws up…splicing thing…i.e., protein, protein splicing. T-cells in immune system apparently recognize not only the parent immunogen but also recognize peptides that get sliced together but that are otherwise unrelated. So your approach to immune therapeutics at the T-Cell level (MS and diabetes), they now have to think beyond insulin because immune system is basing attack on other slices and variants. This creates a whole new magnititude of options for therapeutics.

- The pathology of the various prion diseases, and this is something that puzzles the researchers, are quite different. You have something like chronic wasting disease that is horizontally infectious, you have CJD which is spontaneous or acquired or familial. The brain slides of all the various diseases all look quite different and how is it that when you inject a mouse with scrapie, it's brain looks like a BSE brain not like a scrapie brain. This is all very puzzling. Given all this, it is quite species specific in terms of the malformed prion proteins. The work needs to be concentrated in BSE and CWD. Maybe we could see how some of the species things differ and use better tools to get there. The study opportunity we have in abundance is CWD.

- Still don't know if we have actually described the infectious disease. The more we look at the Japanese infections, we may find some "noise" there. There may be infectious diseases we haven't described yet. Believe BSE is a mutation of a prion that hadn't existed before 1980. Did it come from scrapie? Did feeding sheep offal to cattle cause this problem…or are there other diseases out there and amplified by feeding cattle to cattle?

- BSE is dose related - a low dose may only affect animals that are genetically susceptible. A high dose will result in BSE infection and overcome any genetic factors.

- As far as we know, there is no relationship between a mule deer CWD and TME and scrapie and BSE. But more work has to be done on this with more isolates. There are number of groups that are looking at host range for CWD…also work being done in Wisconsin and at the National Animal Disease Centre (NADC) looking at raccoons (Dr Femer) and there was a comparison with inter-cerebral injections. They are finding that they developed TME rapidly, scrapie took longer and they haven't come down with CWD yet. NADC, Colorado Wildlife and Univ. of Wyoming looking at cattle susceptibility inter-cerebrally, orally and by contact. There have been cattle inoculated with the Whitetail deer isolates (also at NADC as well, I think). Cattle, sheep, goats and others also being looked at.

- Is there any relationship between chronic wasting disease and BSE? Probably not. Looking at cross-species transmissions and other issues, want to improve answers which are now… "probably."

- Work over the past year has revolutionized the whole concept of infectivity in muscle. It has appeared in the muscle, not just the nerves going to the muscle. It would not surprise me if someone comes up and publishes that muscle taken from BSE infected cows contain the protein. This conjures the need to study or confirm that prion infectivity is not transmittable through muscle and blood. While UK studies indicate there is no muscle infectivity, the results are inconclusive due, in part, to the limited number of animals included in the study. In recent work, some unpublished, (for the first time) muscle has either been shown to contain the pathologic protein, or in the case of Prusiner's mice, infectivity. That includes that mouse model, it includes the Swiss demonstration of PrP in sporadic CJD muscle and also for variant CJD muscle. Furthermore, a pathogenesis study in hamsters using the 263K strain of scrapie, has shown the presence of protein appearing about halfway through the incubation period and rising rapidly during the clinical phases of the disease at which time a bioassay was done and there was two to three logs of infectivity per gram.

- Along with muscle tissue, blood needs also to be looked at as an agent of infection, but at a lower level of urgency than muscle. The reason for that in the United States and perhaps in Canada, blood was one of two or three things that were exempted from ruminant to ruminant feed ban.

- The limited range of BSE infectivity so far detected in cattle is an interesting observation. So far, there is no indication that anything beyond the tonsil and perhaps the blood marrow is infectious during the incubation period. It shows up (post mortem) in lymphoid tissue in the distal ileum, but why not the spleen and regional lymph nodes. We know that it is there in sheep, but we don't know in cattle. We need repeatable tests using sensitive techniques, including bioassay. There is a big difference in saying that something is not infectious and saying it's either not infectious or has an extremely low level of infectivity. Clearly, pathogenesis in cattle and elk need a good deal more work.

- Believe there is a need to establish minimal TSE doses in primates required for infectivity; that's as important as the phenomenon of trying to establish a minimum dose for BSE in cattle. So I would like to see the same kind of data requirements for BSE and CWD in primates. I'd like to know just how much of the species barrier exists.

- The whole genetic field is wide open; whether its classical genetic selection, or genetic engineering, it's absolutely wide open. There is some interesting work going on in Korea on creating a GE cow resistant to BSE (according to Reuters: Professor Hwang Woo-suk at Seoul National University led a team involving Japan and USA).

- Dr. Don Knowles is looking at genetic resistance factors in different cattle breeds in the US (Pullman Lab in Washington State, Animal Diseases Research Unit, Agricultural Research Service, USDA). They have tried this unsuccessfully in England, where there are many breeds, so I don't think he will find anything. While this is fairly routine research work, if we find variations, it could change everything overnight.

- There is pretty clear evidence that protein alone will not transfer disease. You need another agent along it... it could be RNA? In previous work, it was probably RNA contamination which allowed the protein to fold other proteins.

- Pathogenesis is clearly a priority area of study. Here the question is what is at the root by which infectivity gets from the gut or the skin or the blood into the brain. Here a major player is Adriano Aguzzi looking at which cells are attacked first, and the sequence by which cells get infected up to the brain. Other trusted names in disease pathogenesis include Corinne Lasmezas, Dr. Ray Bradley and Dr Roy Jackman.

- On the topic of etiology—whether misfolded prions can induce the disease by themselves—Prusiner has found evidence that is the case, but we are finding other opinions. My opinion on the prion hypothesis is that it is the best thing around. It is an intellectual umbrella that most of us in the field feel comfortable working under. The idea that there is some sort of poorly perceived virus or some other infectious agent like a bacterium that's hiding in these preparations is, I think, ludicrous. If Stan Prusiner isn't right, it has to be something almost equally weird to explain the data. Although the prion hypothesis has not been proven, it is a good hypothesis with which to design experiments around. I am comfortable with reserving final opinion for the future. How you go about establishing if it is a protein only agent, or whether there is something else… I think that this is what science has to do, the progress that has to be made. Absence of proof is not proof of absence. We might find something tomorrow, or it might go on for another 25 years until the actual steps of prion protein conversion are completely elucidated. Many people don't realize that this is tough stuff…getting to an answer will take a lot of time and money; there is no quick fix.

- The general observation is that you can inoculate cattle and infect them intercerebrally with scrapie, but infecting them orally with scrapie is extremely difficult. Other studies suggest that the bovine can perhaps support more than one phenotype of prion disease. It is quite interesting that only in the last year or so other countries have come up with claims that they have possibly identified other strains of BSE such as the Italians, French and Japanese. This requires the UK to go back and revisit some material using new methodologies to see whether there may be sub strains of BSE there.

- We've done one large pathogenesis study which has essentially been running for 13 years. This is where we sequentially killed cattle that had been exposed orally, to their tissues and inoculated them first into mice, and later into cattle, to try to find out where infectivity was distributed around the body. We quantified that the mouse was approximately 500 fold less sensitive than the bovine. There are one or two developers of transgenic mice who would claim that they are more sensitive than using the bovine, and time will tell.

- We really need to focus on Peyers patches. We have found a trace of immunostaining in the enteric nervous system, which might indicate that the entire gastrointestinal tract might be a small risk, but the bulk of it is certainly associated with Peyers patches. They are not uniformly infected; not every Peyers patch will be infected and not every follicle within every infected Peyers patch will be positive. It highlights the complexity of trying to use a different type of rapid diagnostic test in the abattoir to test the ileum rather than to test CNS. With our research so far, the indication is that there's replication in the gut initially and then it appears in the CNS… and we don't know how it gets from A to B. We suspect that the autonomic nerves are involved, we have one trace positive result on bone marrow which was at 38 months after exposure, but we have not found any infectivity in that tissue at the time points before or after that one that we collected. And we have had one trace positive result in tonsils which seems to suggest that there is possibly a lympho-reticular involvement, but to a much lesser extent than you see in sheep. But we would need to firm up on these things using more animals, more time points and more sensitive assays. Sampling is a major problem. If you miss the right positive follicle, then you miss it.

- Pathogenesis and specifically pathogenesis in the central nervous system (CNS) is the priority issue: when does it arrive in the CNS, when can a rapid test detect it. This could be resolved in the space of six to 12 months because the materials are available, they just need to be accessed and tested, but which could have ramifications worldwide.

- The recent result from a Swiss laboratory, where they detected evidence for infectivity in the muscle of humans affected by sporadic CJD is work that should also be considered for BSE. They had done some preliminary work in cattle and found nothing so far. If something does turn up, then there is a need to try and quantify that risk for the consumer. And if there is any risk in peripheral tissues, and particularly muscle, then it is likely an occasional risk and one that isn't likely to generate a significant danger of humans. Stun guns that have been used in North America have the potential to mix brain parts with tissue. I am keen to pursue pathogenesis as far as I can so that I am confident I know what is happening. I'm still uncomfortable about the fact that I really don't know how it enters the body, I don't know how it gets from the guide to the brain. But I also feel that just leaving it to occasional researchers to do, to take little snapshots of the pathogenesis using their specialized models is probably insufficient.

- Evidence on the minute amounts of materials required to cause infections brings attention to transmission through contamination of the farm environment.

- There are no improved strain typing tools on the horizon at the moment. We are at the stage where a whole range of tools are being used, but none of it is being brought together because there is no mechanism for bringing people together and forcing them to divulge their pre-publication data to each other. They don't see it as a global crisis. It is always the crisis of individual funding.

- Purdy and others are looking at treatments for organophosphates, mineral imbalances, or exposure to bacteria and autoimmune diseases, but none of them fit the epidemiological picture. I wouldn't be surprised if organophosphates, for example applied to an infected animal possibly compromise the animal, possibly precipitating clinical signs, but not spontaneously generating BSE. Certainly the work that has been done in scrapie over here has looked at soil structures and mineral content relative to the incidence of scrapie and does not show a correlation to manganese and selenium levels. We're dealing with an infectious agent at the moment but whether prions protein alone as opposed to prion protein and protein X or prion protein and nucleic acid… here I've still got an open mind. And I look at it fairly simplistically from the position of needing to control the disease. And I still feel you can control and eradicate it on the basis of epidemiology, but I would really like to know more about the agent to reassure myself that we have it right.

- I am not convinced that the BSE issue is food related. Some 15% of CJD is familial (genetic) and 85% is spontaneous, but it is rare. But if you have a rare disease in humans related to a prion like BSE, why wouldn't you get some somatic mutation that would lead to this disease in cattle in an isolated instance or an occasional animal is going to develop this. They have tested 2500 in Alberta in and around related the first case, and we didn't come up with any other examples. In the UK, it is clearly food related…they probably had closer to 1 million animals affected

- In the 1940s and 50s there was evidence of spongiform encephalopathies in the mink population (MSEs)…they were fed culled animals. This predates BSE issue.

- I just believe that anywhere there is sufficient number of cattle, you will have spontaneous cases. Weybridge and CFIA looked at the Canadian BSE case and didn't see anything different from the UK cases. There is a real issue with that…if you have a mutation that affects the germ cells you will be able to pick up that mutation in every cell. But when you have a somatic…say a mutation that occurred within a prion within a brain cell…then the prion protein will trigger the disease. But then most of the prion protein you detect in the animals after that will be the natural prions because they are being produced by other cells and gets precipitated out because of the presence of the abnormal protein; your normal protein will fold the wrong way. So any time you do a sequence on these even with a spontaneous mutation in a somatic cell you will come up with the same sequence as everybody finds.

- We just haven't been screening all downer animals; if you do, you will occasionally find a mutation. That is problematic if it is somatic. In UK, you would expect them all to have the normal prion protein when you sequence this because it is triggered by a tiny amount of food. If it is all the normal prion protein folded the wrong way, you'd expect them to all have the normal prion protein and they do. Scrapie was around and people were doing research on scrapie and picking up the spontaneous one. Because of the OIE regulations, countries are afraid to look for these. Nobody wants any more… you might find mutations. Italy thinks they have something different in BSE…reporting that they might have found a mutation; some of the milk replacer fed to cows in Japan came from Italy, raising some concerns. It is important to sequence these genes anytime you get a spontaneous or sporadic case of BSE.

- Purdy from the UK and his environmental views creates another perspective. Non-infectious causes have been postulated and Mark Purdy has been one of the most vocal advocates. The link between environment, copper and manganese, and the structure and function of the prion protein is still very, very poorly understood. It is true that mature PrP is a metalloprotein; it binds copper *in vivo* and possibly other divalent cations as well and it has been show *in vitro* to in fact alter its conformation in relation to experimental adding or removal of divalent cations. But moving from there to what he thinks is actually the initiation of a neurodegenerative process based on--I believe he is one of the people who believes it is initially a disturbance of redox metabolism and metal metabolism in the brain-- I think that is a long jump. I'd love to see more research on the question, but so far I have been unsatisfied with the rigor of the questioning.

- I believe in 2000, there was a classic paper published. They compared the convertibility of different combinations of PrP fibrils and recombinant PrP from the (sort of) post species. They found that the convertibility of human PrP by cervid PrP aggregates was roughly the same and quite low as the convertibility of human PrP by BSE in aggregates..

- An understated researcher is Dr. Moira Bruce at the Neuropathogenesis unit in Edinburgh. Her work was one of two main streams of investigation that led for instance to the *de facto* clinching of the connection between BSE and vCJD. Along with their publication was that from John Collinge's group at Imperial College in London. Byron Caughey is a stand out and that group in Hamilton, Montana with researchers including Richard Race and Suzette Priola who are all strong contributors to the field…they are very much focused on the practical issues that CWD presents. I would also have to add Neil Cashman to the list is an excellent example of a Canadian who has made global contributions most recently through his work with rational production of anti-PrP res? antibodies. People originally involved in the UK CJD surveillance system such as Robert Will and James Ironside stand out here. The first identified vCJD and they have become research powerhouses on the human side. They are doing ongoing research in epidemiology and pathology that have enlightened various aspects on the practical side of the human disease. Paul Brown is another standout from the National Institute of Health, who was involved all the way back to Charles Gajdusek when they were first doing the primate inoculation experiments, proving that TSEs were infectious. His focus all the way along has been on medical safety, back to prion diseases, blood and decontamination and so on. His research has recently shown that pressure treatment (as opposed to heat treatment)…modest temperature, but high pressure, and have a very strong in activating effect on prions. He is proposing that it actually tends to preserve the desirable characteristics of things like feed and possibly meat (may be anti-prion treatment for meat). It may be something as well, that in the right environment, could in the right environment inactivate prions the on surfaces of delicate surgical instruments. Calgary Biosphere is a group that have developed a chamber with 150 psi and 150 degree Celsius, that they think inactivate prions. But the testing process to be sure that is the case is quite long. This is something that Canada and the US really need. We need facilities that can handle the validation work, the bioassay validation work, to see whether these processes are really effective. The scientists that will be knowledgeable enough to handle this kind of thing, do not tend to gravitate in large numbers towards that kind of highly applied research. It is what you call technical research because you are not so much investigating fundamental natural processes as you are basically trying to understand the parameters of something that can be applied directly.

- Its relatively true for all the TSEs, we don't know how it gets to the brain. We can find it in lymphoid tissue and in scrapie and CWD, it stays in lymphoid tissue. In BSE it is transient, and we have no idea where it goes… scrapie has been successfully transmitted via blood; others think that it goes from the lymphoid tissues right into the CNS and then retrograde to the spinal cord and the brain. But we just don't know.

Diagnostics and Testing

- For the most part, a good diagnostic tool will lead to management approaches to halt the persistence of the prions in the system. Detect where it is, design management practice to minimize exposure and then minimize risk…lower than where it is now. This disease is a very minimal impact disease and does not come anywhere near the infectious diseases in terms of potential impact and importance. It needs to be resolved and solved…however, once a good diagnostic is in place, it will put us in a position to minimize the transmission of the disease which is pretty good for a low transmission disease.

- There are a couple antibody tests that are approved in Europe including Biorad and possibly Roche, but of course these are on dead animals. There is a proposal to the University of Alberta for people in engineering wanting to do miniaturized tests on a micro-fluidics chip and that might be applicable to a live animal. But the problem with a live animal tests is that it is very hard to separate the wheat from the chaff. Live animal tests have a long way to go and need international recognition to mean anything.

- With regards to the lab on a chip…microfluidics platform is there (with money), it is the biology and chemistry that will be the issue. Can we deliver a live on-the-animal chip in three years…hope so, but tough to say without a live animal test and to know what is and isn't hocus pocus.

- There are numerous groups involved in rapid testing and there are currently three on the market. BIORAD is the test we have adopted. There is Prionics out of San Diego. Ayerst is another. Some of these are ELISA-based and others are Western Blot based and that is a whole other world. Companies are working with Universities and publicly funded groups. Have to look at both private and public sector.

- Immuno-histo Chemistry is the gold standard test with a 4-5 day turn-around. Rapid tests are 24 hours. Expect new technologies. 2-3 people working on a blood test…it will happen and it will be the big breakthrough. That has always been the great breakthrough on any neurological disease; not needing tissue. Now have blood tests for Parkinsons; can look at enzymes in blood that breaks down. Prostate cancer – blood tests are based on chemicals that are released or produced from cancer cells. Blood tests will be looking for anti-bodies developing against the prions or the prions themselves, but from what we believe, they are not floating around the blood stream.

- In the area of diagnostics, it is just a matter of time before someone finds a much more efficient, less costly and invasive way to diagnose BSE and other prion diseases in animals. Unless we're in it now, I would be less than enthused to see a new diagnostic service developed in Alberta based on current approaches such as of extracting and grinding brains and inserting into mice or using any of the assays used in finding prions.

- I think we need to have someone to push on the development of a diagnostic and provide the resources and the opportunity to move that forward. It depends on the person you recruit whether they would have the ability to capitalize on what is out there in screening tests.

- The private sector is trying to look further into the diagnostics, but it is important to have someone watching who is doing what and then maybe making a deal to make this happen faster. For government, agriculture, ranchers, etc. when we are in the vaccine business looking at particular problems like Denge fever, things would move slowly because there was little money attached to a solution. They agreed to all put some money on the table for a preliminary research, and then others could pick up from these, that would be another approach. But we need someone from another aspect who can link up with them and get up to speed on what is happening and maybe get support from government (someone coming from a unbiased third party). This could be a public-financed scientist to help speed up the process.

- The diagnostic area is well-equipped in Europe. Canada has very little diagnostics. Most labs in Canada are going with Bio-Rad with the ELISA test, or Prionics. They are both ELISA tests and for sensitivity or specificity, don't know who is best. There is one US company that is going to work with the UK that is using a capillary test (similar to ELISA test) that you can use in the field. They cannot speak until they get approval from the British Council. My understanding is they use brain and lymph node but it is a faster test and more specific test. Use a needle that goes into the base of the head. It is a biopsy so it is faster and the animals can be alive.

- Diagnostics is a key aide in progressing in other areas especially pathogenesis. Do we sit back and wait for private delivery? One of the directions Congress gave to the Department of Defense is that they wanted an animal diagnostic. The medical panel recognized the need for better diagnostics. The committee however felt the need to broaden out the direction that was necessary. They did recognize the need for diagnostics. There were some really interesting and exciting new things and techniques that are coming forward. Proposals that were coming in from the commercial entities were generally antibody based type of work or TSE diagnostics, some of which have already been commercialized like the rapid ELISA tests… however, they were somewhat surprised they did not come out with any groundbreaking information. What is coming out in the literature, related to more sophisticated diagnostics, like detecting very small amounts of proteins, and perhaps some of this work is appropriate as it comes forward into the prion field. Not everybody may know about this. And there was commentary about other kinds of diagnostics related to surrogate markers, not necessarily looking for the protein itself, but other markers that might be useful. There were proposals coming forward and there were others applying things like proteomics in genomics to whether or not those approaches might help sort out surrogates that might be useful in diagnostics. There is a lot of room to move forward in developing or doing research work that helps develop better diagnostics.

- There are two aspects to the testing: First, what are we going to need to do in the first week if we start looking at all these 30 month+ old animals…large number tested rapidly. This is the quick and cheap testing with a high level of no false negatives (false positives are not okay). Second is the sophisticated in-lab testing that will be available for fingerprinting the cases and maybe eventually relating one test result to another (sophisticated and sensitive enough say it is this type of BSE and related to this or that animal). This would obviously be the research tool.

- Everyone is racing for a live test. What approach to take? I would not fund anybody who was not working with someone who is already actively involved. It would be nice to do some research to get the far-fetched ideas out there off the table. Another scientist in the UK has research on organophosphates and so on.

- Even in an international community, there are questions about what is an appropriate diagnostic tool. The Japanese tests are actually inadequate for properly detecting intermediate cases. Maybe they can get advanced cases but not intermediate cases. Bottom line, there is a splendid or urgent need and opportunity to develop greatly improved diagnostic tests for prion diseases, that can distinguish between the native precursor prion proteins that is doing is normal job and the abnormal prion.

- All the tests, rapid or otherwise, have been compared with respect to their threshold to sensitivity and threshold of detection always using central nervous system tissues diluted out. And I think it would be important to test them also on their ability to detect the protein in tissue in low titre tissue such as the distal ileum or tonsil.

- Diagnostics are the single most publicly funded goal in our field today. The problem is getting a test that is sensitive enough to do the job. That may come next week or not for 10 years or never, we just don't know. Funding is both private and public. The Department of Defense have spent $45 million this year (note: DoD is administering US National Prion Research Program), most of it for the purposes of developing a screening test. Every two months you hear a press report from somewhere that someone has a screening test, and then you don't hear about it again because they actually don't have one. And so all these little private venture companies come out of the woodwork, and they think they have a shot at it… then they find it's harder than they thought. Proving it out in the field is the second order of consideration. The main thing is getting a test in the laboratory which will detect something in blood; it just doesn't exist yet. In the field is another matter.

- We don't need any more post mortem tests…we already have five tests that are commercially in use and they're all good. What we need is a test that will detect nanograms quantities of PrP, and there isn't any.

- I don't think there's any shortage of tissues to test the tests. We know in rodents, for example, you can predictably guarantee a low level of infectivity in the blood. So anyone who has a test can take a terminally ill 263K infected hamster and know he has infectivity. If he can't detect the protein he's out of luck. Screening test just doesn't seem worthwhile for a new effort when everyone else in the world is already working on it.

- Neil Constantine who works in Dr. Robert Gallo lab in Baltimore has been working for two or three years on immuno-polymerase chain reaction (I-PCR) and making progress. We'll find out (at the upcoming Cambridge HealthTech Institute Tri-Conference, March 24-26, San Fransisco) just how much progress has been made. I know it has not progressed to the point where it is a viable test but in principle it is the single most sensitive test you could apply. If I were to put my money down, it would be on a test that would first detect PrP in very low titre tissues like blood. This test uses the PCR technique allied with a ligand to antibody to antigen…it is capable with purified proteins like albumen to detect as few as 50 molecules. The problem is that it works best with very purified proteins, obviously because with that kind of technique anything that you amplify that is in the protein is going to mess up your test, and that is the main problem of using it on serum. But again, it is just a question of technical advances, separating background noise from what you want; in this regard, it is probably doable.

- A deliverable of a Canadian network (involving CFIA and Health Canada along with a late-joining member from Genome Canada), through a CIHR grant (through Safe Food and Water Initiative) represents an attempt to develop an antemortem test for BSE, the Holy Grail that so many are aiming for. In all, there are three tests that we put forward in the CIHR grant. One of them is an in vitro conversion test that we developed (that is published in the Journal of biological chemistry in 2002). Another one is based on the development of prion specific antibodies, similar to the antibody that we reported in Nature Medicine in June 2003. We also have a completely novel new test invented at the University of Toronto, based on a notion of how prion proteins converts in disease…we have been validating it in the lab.

- A second study involving 300 animals was primarily established to collect banks of tissue and fluids, samples from live animals or postmortem for the evaluation of new diagnostic tests. The UK was aware of efforts being made to produce live animals and postmortem tests that were better than the ones they had, but they did not have a bank of validated tissues that could be used to evaluate the performance of these tests, especially at the preclinical stage. So 100 of these animals received a dose of 100 g orally; another group received 1 g and the other group were unexposed controls. Groups of four or five were killed sequentially along with controls, starting in 1998 and the study is almost complete. It means we now have a vast number of tissues and fluids banked in freezers that may be of value for the evaluation of diagnostic tests.

- The UK in general, and the VLA in particular, are involved as a community in OIE reference laboratories in evaluating some diagnostic tests and this is where the big demand is coming at the moment where everyone sees a large market out there, and clearly want to get their tests evaluated and approved. Unfortunately we're working with a very limited resource in terms of the amount of brain material that we have stored in our freezers that is of any value to the evaluation of these tests. The more we use just to evaluate tests, the less there will be for further research. We are being a little cautious of our use of it at the moment.

- Regarding antemortem diagnostics in the development phase, I haven't seen any data yet that would give you the degree of confidence in the outcome that would enable a government to actually implement them for screening of cattle going for slaughter.

- In relation to BSE, everyone is going to the find serious difficulties in actually finding materials to evaluate diagnostic tests in the longer-term. The UK deliberately infected 300 cattle for materials, however, this was a massive experiment costing around £1,000,000 a year, and it is still a very limited resource. Anyone who comes along with a breakthrough is going to be faced with this challenge in future. It could well be too late for the live animal test in bovines.

- The UK has invested in infectivity detection since the early 1990s…they were looking for alternative markers and biochemical markers to see if there were any physiological changes that could be detectable that would be associated with BSE. They have so far failed to detect such changes. There was a new raft of proposals that was funded by the joint UK funders starting early 2003, particularly targeting the new spectroscopic methods of mass spectrometry and infrared spectrometry, in association with some sophisticated computer programs. These seem to be showing some potential, but with all of them, the key is to educate the computer with enough data from known BSE positive animals. And again were back to that limited BSE resource and the degree of confidence that you want in the use of that test. If you're just looking for disease control on the farm, then you can use the a test that is 80% sensitive than specific and you can eventually get ahead of the disease. But if you're testing animals going into the food chain, then you want to be closer to 99%. Sometimes governments call for tests with 100% specificity and sensitivity. You're going to have great difficulties selling anything with slightly inferior performance to consumers even though realistically they might be much better tests.

- People tend to think that the cost is there in the test, but the real cost is in the collection and transportation of the samples and the infrastructure you have to put in place. The cost of the test is peanuts in comparison to these costs.

- Now it is possible that the gradual development of transgenic models may give us one gold standard that is universally susceptible. That's a dream, that may never happen but it may lead us to a panel of transgenic models coupled with the wild type models, where essentially susceptibility to X out of Y…or X models of mice to Y isolates could actually give you a fingerprint for a particular strain. But at the same time, there are various strands of research, some of them are involving enzymatic characterization, looking at cleavage site's… how different strains behave to different treatment processes using these enzymes, and their subsequently immunostaining the peptides that you get out of there. Others are using mass spectroscopy to try and characterize those peptides. At some point these pieces of jigsaw I think will come together. Now it is conceivable that someone will just make a breakthrough and leapfrog all of these pieces of jigsaw and just come straight to the answer, but I don't think we are there yet. For example, there are those like the California group that are working on using their conformation dependent assay. And they've used it on isolates of hamsters scrapie, they've been able to separate them. And they've been able to separate scrapie and sheep and BSE and CWD in elk. But it is still a relatively crude approach at the moment. But once they get access to enough material from natural cases, who knows, they may come to the point where they can actually define each one. It may well be that this is a myth anyways. We have the confounding factor in sheep of the genetic involvement, and the extent to which that influences susceptibility in phenotypes. In cattle, who knows, it may turn out to be one strain of agent… a very limited range of genetic conformation, but which generates just an occasional case that looks slightly different. So maybe the genetics will show up one or two surprises in the future.

- Cell cultures have been difficult to work with prions diseases. One or two breakthroughs recently where they have been able to produce genetically modified cell cultures, show some promise. Once you can work with the cell culture, then potentially you could start applying spectroscopic methods or electromicroscopy to cells within a few days of exposure to your isolates, rather than waiting for potentially three years to say that this is not infectious… which is where we are at that the moment. So, I think there is room for a big push on cell cultures, we have to get away from inoculated animals.

- Tissues are available but release is subject to the approval of an external committee now, that was introduced just over a year ago, so applications are generally reviewed and this committee is an international one that requires a certain amount of detail about the proposal, what this tissue is going to be used for. Generally our intention is to release where we can, so we try and make sure that when a proposal comes forward we actually clarify most of the detail that is likely to be required before it gets in front of this committee, so that it is not rejected out of hand. In that sort of scenario materials could be provided to someone else to do the work somewhere else. An alternative scenario would be for it to happen somewhere in the world as a form of collaboration, where both parties gain. In fact the whole principle behind establishing the TSE archive in 1996.

- The microfluidics work that is going on in Alberta provides sensitive tests, but I'm a bit concerned with the idea that within 5 years they will have a blood test. Microfluidics is an excellent technology and new, however, maybe they need to go to UK and prove it on diseased tissue and then look to increase sensitivity. You might want to see if it works on CSF…if they can get something that can work in blood, that would be great.

- A priority concern is the inability to diagnose the disease at a point in advance of death, and in advance of an animal's having established a gross abnormal prion count which could represent a threat to humans. It is from the absence of such a test that derives the paranoia and overreaction of nations to countries such as Canada.

- The inability to detect the agent in feedstuffs is another priority. It is that inability that drives meat and bone meal bans. Because you can't detect contamination, you assume every load that contains ruminant MBM is at risk of prion carriage. The result is a massive redistribution and lowering of value of an essential by-product of the industry.

- Adriano Aguzzi in the University in Zurich has done work that indicates we are very far away from relating prions to lymphocytes. There might be some connection to it, but even in the hamster model it is not clear. And for BSE is even less clear. It is very preliminary work and really basic research work.

- Research on the structural features of prions is highly in demand. This will reflect in the end as far as diagnostic techniques are concerned. However, we are talking about a disease with long extended incubation periods. The Rocky Mountain lab in Hamilton Montana, or the other NIH laboratory in Bethesda, Maryland are very much involved in this kind of research for 20 or 30 years already.

- Drawing a CSF sample is not an attractive screening option. What do we know about it being an effective marker in BSE… there are issues with both sensitivity and specificity in humans. There are cases of genuine CJD that don't tend to, on a single sampling, produce visible banding on the western Blot. There is a growing list of non-CJD conditions where rapid neuronal death is presumably the common underlying factor that give varying strengths and signals (positive). There is always that kind of issue with sensitivity and specificity. That general area of investigation is still wide open for creative approaches.

- A couple things to be aware of: one of them is that brain proteins may leak into the blood. Either they get there through the CSF as other proteins do be because there is traffic between the brain and CSF and between the CSF and the serum. It is conceivable that disruption of the blood brain barrier in relatively advanced neurodegenerative disease leads to leakage of brain proteins into the blood itself without the CSF as an intermediate compartment. There is every possibility that brain derived pathological markers will find their way into the blood and maybe ultimately into the urine. The ease of doing sample acquisition to do these hypothetical screening tests may not be as bad as we think. And the other possibility is that there are things going on in the periphery that are not directly brain derived but still clearly could serve as very useful markers. There was a study published a few years ago by a group at the Roslin Institute in Scotland where they found an essentially unbiased blind screen…a brute force approach at looking at mRNA changes in the blood. They found a small molecule that proved to be a chaperone for a hemoglobin maturation and it is a marker of particular stages of eurytheroid precursor development (protein called EDRF); that protein is being looked in the UK as a potential screening tool for blood donors with the conformatory component of the test being based on the prion protein perhaps.

- Regarding prion protein in the urine…it is a tricky thing…there was initially a flurry of interest in when Ruth Gabizon from Israel published their work a couple years ago. It initially appeared to mark the existence of pre-clinical infection or disease both in humans and in a couple animal species (believe there was a hamster model and maybe a scrapie model included in their panel). That result has not been easy to reproduce in other labs; the interest has waned considerably. When I was thinking of urine as a substrate for testing, I was thinking of other markers that may show up…the PrP is not unusual with respect to the initial flurry of interest and the rapid tapering off of interest as others people try to reproduce it and have mixed success or fail outright. This is a common phenomenon in bio-marker research---initial excitement and the flag goes up and everyone thinks we have the answer and then reality sets in.

- On the prophylactics side, if you find you have the ability and mandate to see actually screen for pre-clinical disease…it is almost essential that you then have prophylactic kinds of therapies or attenuating kinds of treatment to offer the people whom you've just subjected to the screening process. You have to look to a therapeutic intervention. I have some skepticism, as it applies on the human side, that it is not going to turn out to be practical on the commercial side or in terms of efficacy. On the animal side you're probably going to find even a smaller market.

- The pathogenesis and diagnostics work together…you can't take these brute force approaches that can actually sift through thousands of molecules and look for their correlations without knowing anything about pathogenesis. And then when you do see molecules you can ask a question—what's actually going on, and the reverse is just as obvious.

Epidemiology and Surveillance

- Really critical for CWD and maybe less important for BSE is being able to detect infections at low levels…bioassays are clumsy and not satisfactory, so detection is very important if we want to go anywhere in epidemiology and other areas.

- There is now some discussion about a different strain of BSE in Japan. I believe that CWD is quite different from BSE - the pathogenesis and epidemiology are different. If you back up and go to molecular pathogenesis, I suspect there is a core mechanism by which the TSEs work, and I suspect that there will be major similarities there between scrapie, CWD, TME and BSEs, just because there are similar lesions produced, and slow progression. I suspect we are going to find great similarity when we get down to the basic molecular pathogenesis. However, at the individual animal level I'm not sure you'll find much information by studying CWD pathogenesis as it relates to BSE. And, if for example you could transmit CWD to cattle, it is unlikely that it would come out as CWD in cattle. Information on strain typing, is something that would be very interesting in CWD. There is a lot more planned for this. That would be nice to know if we are dealing with one strain in Saskatchewan and see if it is the same as what they have been Colorado or Wyoming. Those are pretty important questions to ask when you're looking at epidemiology. If there are different strains might there be different susceptibilities and this might relate to cattle and BSE. We don't have evidence that it is transmissible to cattle, but as scientists and people that recognize we live in the real world, we recognize that these questions should be asked and should be looked at.

- Genetics clearly plays a big role in susceptibility, but the exact role of genetics in influencing susceptibility is not absolutely known. Regarding scrapie, there is a lot of polymorphism in the gene and that does lead to different susceptibility, however, in cattle there doesn't seem to be much of a susceptibility issue. You can look at this different ways. One, you can look at how the genetic background of an animal, the prion protein might interact with an abnormal protein, and how the structure of that protein might influence the conversion to take place. There is a very basic question that can be looked at there. If we look at genetics of species across the population, we might try to look at different genotypes and resistance. So we can look at it at a very basic level and then look at it at an applied level in populations, to understand if we might have resistance or susceptibility. At this time all the people that got vCJD had one genotype, however this might not tell us about susceptibility, it may tell us that there is longer incubation period required for some genotypes.

- Retrospective epidemiology is another area of interest. I'm sure there are people in government doing this. But maybe you can get the detectives talking to the scientists to see if there are models that can come out of there. Linking sciences is certain to achieve benefits even if it is just cross-pollination of ideas, rather than on the ground collaboration. The answer to BSE may come from variant CJD, CWD, or another area.

- There will almost certainly have been 2 to 3 million cattle infected in the UK over the period of the epidemic, most of them consumed. It's still difficult to translate that into an epidemic of so far 140 variant CJD cases. It could be that those individuals are particularly susceptible because of their genotype or some other factor. They may have simply been exposed to a minority strain. The UK has been putting two and two together simply because they are contemporaneous when in fact there is another possible source which might well have been bovine, but it may been a different strain. A pathogenesis study involving only 40 or so animals provided ultimately too few animals to firm up the chronology of this pathogenesis with any certainty.

- That is an area that is still of interest particularly in North America because of the CWD dimension. The possible concerns that CWD might have gotten into cattle certainly raises some concerns. But then you have to look at the alternative route of BSE getting into elk and deer. I'm not saying that will happen because we have not seen any evidence so far of BSE getting into sheep, despite the fact that sheep were definitely fed meat and bone meal. But you certainly have cervids that appear to be prone to horizontal. transmission, and possibly exquisitely sensitive to exposure to some other agents.

- There are still some questions on epidemiology…the evidence for maternal transmission is very slight. But we are at least going back to having a look at the genetic makeup of cattle. . We also have little evidence of horizontal transmission, but this is solely epidemiological evidence that we have not tried to quantify.

- Partly, there are various reasons and most of it has not been proved, but it is the combination of epidemiologist and pathogenesis data that we have. First, the most heavily exposed cohort in the UK would have been born in the winter of 1987 or early 1988. And there is no doubt that at that time, quite a high proportion of consignments of feed contained meat and bone meal that was also infectious. And a risk to the farm of actually buying in infectivity depended in part on the size of the farm. So the bigger the herd, the bigger the bulk order, the greater the probability that they would buy material that was infected. They could be buying every week, but there is no guarantee that every consignment was going to be positive. So it could well be that a cohort of animals born in a particular week maybe have been exposed, but subsequent cohorts may not. In addition if you're buying in bulk, there is no guarantee that it will be uniformly contaminated. But I think one of the biggest factors that has to be absorbed is that the vast majority of animals that become infected have been slaughtered and consumed long before they get to clinical onset.

- If I had a live animal test I might think differently, however, given what we know now about susceptibility to low dose and given the nature of worldwide trade, it's very difficult to always look over your shoulder and know what's coming in behind you. Other countries may not have owned up to having disease… and the risk is coming in several years before you'll ever find your case of BSE. Europe was, from the geographical risk assessment done in Europe, the majority of European countries were exporting BSE before we knew we had BSE. So it is like a Trojan horse, and inevitably everyone has brought in their risk from elsewhere, and all from countries that theoretically did not have BSE, until it was too late.

- CFIA is also involved in assessing of the true natural history of BSE in Canada. They are investigating the imports on a one by one basis geographically and temporally with the conviction of the Cluster Hypothesis…it flows from the belief that renderers, feed mills and producers group in clusters for geographic and economic reasons. An infected import that fell into a cluster and ended its life there would be taken up by the cluster's renderer, processed and redistributed in the largest part back into the same watershed. Only a small percentage of feed would leave the boundaries, in the form of specialty products of high unit value per unit weight. Prions would carry no more predilection to move into those products than into the massive, low unit value per unit weight products which en masse would reside within their own clusters. This is magnified into consideration of East and West as independent clusters separated by the Precambrian Shield in SE Manitoba and NW Ontario. While lots of cattle leave clusters, that's not the issue. In extra-cluster cattle movement, the issue resides with whether the moved animal, by the time of its attrition, will have reached the point of abnormal prion growth to represent a threat to the feed chain. So most of the movements are a non-issue, as they focus on the lesser ones that are. The CFIA is allotting person years to pursue the surveillance redress and the natural history with cluster emphasis.

- Regarding interspecies TSE roles, a question arises as to whether any elk were fed MBM, irrespective of Western Blot indication of classical BSE in the cattle?

- The control mechanisms (of BSE) were independent of an understanding of the capacity for variation. The UK BSE epidemic is an excellent example of how a novel conversion, whether scrapie or some spontaneously occurring prion disease in cattle. Novel variation led to a cataclysm. I am concerned that we are not taking adequate account of the potential for BSE as it is found in North America and other agents like CWD to change in that kind of way that can present you with a substantive new problem.

- A priority at this moment is how do we meet the challenge of ensuring adequately intensive surveillance of BSE in the Canadian cattle herd. How do we actually accomplish that goal technically and logistically, and understand the biology and epidemiology of BSE that we have confidence that we are sampling intensively enough?

- One thing that needs to be done is a case control study of a large series of BSE affected and non-affected animals. Canada doesn't have that resource; the UK and European countries do. I know that John Williams at the Roslin Institute is undertaking that line of research in the UK. If that research pans out, and there are susceptible alleles identified through the case control and genomic scanning approaches, those results could be extrapolated to characterize Canadian animals as well even though we don't have the numbers to do the primary investigation ourselves.

Prevention, Therapeutics, Vaccine, Control

- Vaccine approach…because they don't view BSE as infectious…countries likely won't support vaccine route which means introduction of antibodies that relate to the disease to be more widespread…probably causes more problems than it solves. The diagnostic that will emerge will likely be one that identifies antibodies to the prion.

- Simply improving cattle feed as we did way back in 1997 or 1998, that's an important part of the process, that will reduce the incidence of TSE, but it does not do anything to reduce the risk of spontaneous TSEs. Cattle feed will be important, making it transparent, that will be important along with traceability. Pixas Genomics…partner has technology that was just announced with Maple Leaf Foods in which they're looking at traceability of pork. And Canada may be the lead nation in the world in being able to trace pork from the shelf all the way back to the farm and the actual sow that that pork chop came from. If we are doing that for pork, I don't think it will be long before consumers demand that same technology for beef and chicken. Accountability all the way from the farm to the marketplace into the kitchen. At one time somebody gets sick, they will want to trace it all the way back. That is a DNA tracing issue, the technology exists and as the price of that technology drops, we can see that going forward in the marketplace.

- Tests show that 0.001 g of BSE is not displaying infectivity, but that 0.01 g of BSE is showing signs of infectivity capabilities.

- In the UK, we are seeing cows that are coming down with BSE after the feed ban and on farms that have not had BSE. The question of sporadic BSE is back on the table. And so questions arise about transmission through grass. Like scrapie, like wasting disease, there seems to be a vector of some kind. Is it mutating and getting worse? There is that species barrier that will protect us, but it still causes an economic disaster. If we know it can be transmitted by blood, then it changes everything.

- It seems almost as though this prion disease itself exists in different flavors. The protein folds in different shapes, and it appears to result in slightly varying, differing forms of the disease that are more or less transmissible, more or less rampant in their progression, and differing slightly in the symptoms that they cause in the animals. And so whether it's the form of the disease that exists in deer or sheep or is transmitted to mice, it is subtle variations in how the protein looks, subtle variations in the RNA that accompanies it (to fold the protein incorrectly), this may have a big role in how transmissible it is to humans, and how the disease progresses in humans.

- There is an incredible amount of research into how prion diseases might be treated or prevented. Top researchers include Dr. Stan Prusiner, Dr. Byron Caughey (who developed rapid throughput system for identifying candidate drugs that might inhibit prion replication), Dr. John Collinge, and then the people trying to develop immunotherapies or vaccines against this disease. Dr. Neil Cashman has a reasonable profile in this area along with Dr. Hawke and Dr. Charles Weisman who work with John Collinge. Dr. Adriano Aguzzi from Switzerland also is a key individual in this area.

- A key issue for policymakers and researchers alike and a point of debate in the USA and Canada will be whether or not you take out the SRM from all ages of cattle or whether you confine the rule to animals over 30 months of age. The UK introduced the 30 months rule in 1996 based on the observation (with limited data) that if infectivity reached the CNS at approximately the mid-point in the incubation cycle. The mean incubation for bovines was 60 months; so mid-point was 30 months… so all of a sudden there is a rule, the 30 months rule, and everyone assumes that that was based on the extrapolation from their pathogenesis study. The first point that they detected infectivity in their study was at 32 months, and clinical signs were first detected at 35 months. But there is no telling whether other animals earlier in the incubation phase would have shown up with infectivity. Cleary, this issue still needs to be resolved and it could be key to defining SRM rules on a more scientific footing.

In-activation and Decontamination

- Once a facility has a prion, it will always be a prion facility on account of protocol. This is a limitation in deciding where and why you might undertake prion research there.

- When you set up a facility for studying prion research you're pretty much committed to continuing in that direction. So if they do find some way of disposing and decontaminating infected prions, it would be very useful for the development of research.

- One big problem in Britain was the issue of incineration. We need to get some research in the engineering field about how can you have a facility that can incinerate 50 to 100 full cow carcasses. Disposal issues may be better dealt with by engineers.

- Some work recently out of North Carolina where they found an enzyme that appears to be able to destroy prion proteins. Keratinease (used to break down feathers of chicken)…

- High pressure appears to be the only technique that seems to have a hope of actually being applied for materials you want to conserve and preserve, rather than material you want to destroy. Our research indicates that high pressure in the range of 300 to 700 mega-pascals (700 mp = 100,000 psi) may provide the assurance that even previously BSE-infected meat is safe. Commercial application of high pressure of this magnitude already exist for the preparation or processing of foodstuffs as divergent as oysters, cured hams (and deli meats) and orange juice. We managed to get about a three log infectivity reduction in a variety of strains. High input of infectious material didn't provide full sterilization, however, among the continuing aspects of our experiments, we will put in a realistically low input of infectivity and try to demonstrate that it does sterilize. Food products that go through such intense pressure are flavourful and preferred in blind taste testing. And with regards to nutritional value and retention of biological activity, the pressure treatments are also promising. There are groups in Germany and France that are already working on this and will continue to work on this.

- Materials like carcasses and meat and bone meal and other SRMs can all be incinerated. We have seen that somewhere between 600 and 1,000°C is totally sterilizing and generates nothing in the way of emission of gases. Alternatives like alkaline hydrolysis are fine, perfectly effective, however, hydrolysis is far more expensive and bothersome than plain old incineration. And the alternatives of using this material as fuel, which is a form of incineration, or putting it into asphalt… all of these things have been tried and they work.

- I'm not very optimistic on enzymes. I know there is a company in South Carolina using enzymes… and I looked at it fairly carefully and found that they had to treat this up 120 centigrade and then they cool it down and put the enzyme in… I may have missed it, but I didn't see any control in which the inactivating effect of that heat was evaluated and without that you have no idea if the enzyme is adding anything to the process at all. That is why we threw an autoclave specimen into our own work and its fairly clear that high temperature is a major contributor to in-activation… but we have new data to show that high pressure also does contribute. The main contribution of high pressure is to make food palatable… if you autoclave a hotdog it turns into mush; if you subject it to autoclave temperatures under high pressure its very good. In the area of food processing this is where it will find its greatest application.

- Decontamination of contaminated materials. Incineration at extremely high temperatures with air curtains is safe and efficacious, as is alkaline hydrolysis, but is there another, easier way. Lloyd Spencer at ADRI Ottawa is convinced that composting will do the job.

- Incineration capacity is an issue and the restrictions on operating incinerators is becoming more difficult. CFIA is looking at methods of composting to try and destroy prions. Lloyd Spencer, a longtime poultry researcher, is doing work on this and has been pulled into work on TSEs. This will be an issue with SRMs and carcasses if we ever have any more cases.

Other Select Comments

- Need a testing facility and a way of doing research on large animals if some are found to be positive…the level three facility that is going up in Edmonton should cover this off. There might be a need if we decide to appoint faculty member to work on prion disease, there might be need to increase facilities mainly for small animal models, goat, sheep, whole issue of elk is one that needs a focus. It is another issue of containment if you're going to work with elk.

- The EU is building new lab in Paris (European research lab on prion disease…interesting concept; now realize they need to put their expertise in one place and work together on it…rather than a small lab working in isolation…you need to bring all the experts together. This shouldn't take away from the basic science that is going on, because that is the engine that drives the bigger projects…if we don't have these, we won't have leadership. It eventually comes down to leadership and we need scientific leadership to convince them that this needs to be done.

- The Proteomics network is finishing its term after 14 years. They will need to move forward with some other funding mechanisms.

- The Protein Engineering Network of Centres of Excellence (PENCE) have been very strong in Canadian protein work. PENCE is a group of 30 or 40 scientists doing different things. Protein has come of age and everybody is really moving forward with new technology; perhaps PENCE will have to reinvent itself and perhaps moving forward into the translational work—the biological, biochemical and the applied side might be where they are headed. Dr. Cashman and Dr. Westaway are the two experts in Toronto, along with Mike Coulthart in Winnipeg—those are really the leaders in Canada.

- TSEs have been there for the last 15 years, to be honest, North America hasn't paid much attention to this. In Canada we spend no more than $1 or $2 million in research on prion diseases up to now. And I haven't seen much increase in it, but I hope that governments will pay attention. In the United States, the NIH is spending around 25 or $30 million and I'm sure now there will be more investment. Time is right for Canada to at least build capacity to look at animal health and how it relates to human health. We are at a very rudimentary stage despite this protein being so critical.

- Canada's national capacity, with the national lab in Winnipeg, I guess we have done some good work. But if I count on my fingers there are only five or six labs which have capacity to do research in this field. So the question arises if we should have a center of excellence in this field where you can bring the best brains and let them evolve the science. The gap between the time it takes to do in vivo (animal or human study) is a very long process. So that brings up the question of what you do in the meantime… what are the markers one can use… what models can be used etc.

- There is a needs assessment out there that is being constructed on a national level (Canadian) as follow-up to the Sept. 27, 2003 meeting in Edmonton. Priorities were boiled down to include basic science, increased policy and risk assessment and environmental contamination (direct studies in cattle did not make priority).

- Only other piece that is missing is the mechanism to train and retain prion scientists in Canada…they tend to leave the country. In order to attract and retain the best and the brightest prion researchers to focus on Canadian problems… we need training grants for graduate students, post-docs, perhaps even young investigator awards. We need to have Canadians working on the problems of Canada.

- Regarding network development, there was an initial effort to pull everyone doing prion research in Canada together under one umbrella. While there are people interested from various institutions we hope to be able to recruit more. Securing grant funding should assist. The players in this field include DR. Neil Cashman, Dr. Avi Chakrabartty, Dr. Arumuga Balachandran, Dr. Mike Coulthart and Dr. Alex McKenzie. There are some basic prion researchers we hope joint the network. I'm hoping that CFIA and Heath Canada contacts will be recruiting people on the government or agency side. The concept is we know what each other are doing and there is less wasted effort and there is some funding to carry out research projects that are aligned with the goals: define the risk for Canadians and develop a viable diagnostic test for these diseases (human and animal).

- We are looking into the possibility of developing a Center of Excellence in Prion Diseases, including both human and animal prion diseases, and components from basic science to communication. Alberta may be interested in becoming a node in the prion research effort focusing on their priority in the overall scheme of things. We might have one node dealing with human diseases and one dealing with animal prion diseases. The University of Alberta and Calgary could be a part of such an approach along with interested from elk or cattle associations. It makes sense for Alberta to be focused on work related to BSE.

- The US research model is to spend huge amounts of resources in a few places. For example Stan Prusiner's lab has had $65 million funding from the National Institutes of Health (NIH) over the past 10 years. Essentially the Canadian model is about a little bits and pieces—a little lab here another there. A large, well-funded central facility is required in Canada and it is not something that any of us individually have the funds to create…something in the order of $15 million to properly outfit a centre. From a basic science perspective, it makes sense to have a center focused on protein misfolding with application to TSEs and other diseases like Alzheimer's, Lou Gehrigs, and Parkinson's disease, and even certain types of cancer. There is a strong case for a national TSE research facility located in Alberta near a campus with access to all the required elements (expertise in biochemistry, protein chemistry, structural biology, and other areas of science required to solve the problem in protein folding).

- Research facilities should be attached to a university. This is the bits and pieces model that we all work under. The University of Toronto is superb scientifically, but there is very strong protein chemistry and structural biology at the University of Alberta. There is outstanding research expertise in Alberta. So if you have to build something in Alberta, I would recommend looking there. If the provinces and agencies all chipped in, you could build the facility anywhere. Build it and they will come… that is the model of the United States. But you need to attract the key scientific leaders/researchers, nailing that one is tough but a critical component of success. University of Toronto, McGill University, UBC and University of Alberta are possible locations for a centre…all have certain advantages. Structural biology strongest in Toronto, bioinformatics and proteomics is strongest at McGill, Alberta is strong with protein chemistry.

- Regarding the UK research budget, as vCJD epidemic appears to be slowing down so to is the funding into BSE and scrapie. The funding bodies have wanted to redirect those funds to other areas now. The UK has invested in buildings which may only see one generation of experiments go through them.

- Many great researchers from Alberta and Canada end up elsewhere. One exceptionally bright Albertan doing his post-doc in the US has been considering coming back to Alberta. The question then is how do you attract a person like that…yes, you would need some facilities, but it goes beyond that. For BSE, we would have to import some of that expertise and build some young researchers and have a couple people working with BSE. You'd be surprised how quickly we would become internationally competitive. If the one individual I spoke of could come here, we could transform this to the next level. We need to dedicate a space and assign 6 or 7 individuals and soon they will have other researchers and then you build strength.

- CFIA has identified the following critical research issues:
  - Speed and accuracy of prion detection in live animals and animal products.
  - Improved methods to detect prohibited material or prions in animal feeds.
  - Understanding genetic susceptibility and improving the detection of animals to determine which animals may be more susceptible or at greater risk of developing prion disease.
  - Better differentiation of types or strains of prions coming from elk, sheep and other animals.
  - Cross-species barriers regarding prions.

- Secondary issues of concern from CFIA:
  - how and under what conditions prions are transmitted between animals.
  - the role of metal contamination, if any, in transmission and the factors in tissues and fluids, such as blood which may influence the infected the of prions.
  - effective methods for disposal and destruction of tissues and animal carcasses which are infected.
  - longevity of prions in the environment and the need to prevent the spread of disease is to humans and animals and wildlife.

- We desperately need researchers working TSEs; both basic and applied work. We need to have materials and tissues and animals available for research for everyone; there is great demand for TSEs in general. We need infected animals such as deer, elk, etc. to undertake experiments. Transmission studies need to be done. We also need to have BSE (and other TSE) tissue available for method development and test evaluation. We need to have available different strains of prions for research. We need to have available cell lines susceptible for TSE research. All those things are not really existing in Canada. Things get passed among the different research labs, from one lab to another, and approaches are not standardized, so there is a problem with respect to repeatability of results… that is the major area that I believe we need to bundle our energy and develop a strategy.

- Department of Defense in the United States that has put out considerable amount of money for TSE research last year. They are funding 38 different programs in total; 17 out of 38 have the primary focus on this direct development of sensitive and reproducible antemortem diagnoses. So there is lots of work done and of course, in Europe being the center of TSE research for a very long time, antemortem tests are in development as well. We really need to make sure that we don't duplicate what other people are doing already. Especially with those having years of research ahead of us. The EU investment is in the order of €150 to €160 million. The investment there is far beyond anything that we have available, and we need to build our linkages back to this work that is going on and take advantage of it as best we can. Where we would come in is that the validation stages if something is determined to be a potential test, then we would probably want to cooperate in the validation.

- Regarding prion research, there is a tremendous diversity, almost an explosion, of activity since 1996, when the EU decided to fund it heavily. Helping to form a national network of prion researchers in Canada and before too long in North America, would be the right way to go. Through meetings, a consensus has emerged…very strong that Canada needs to establish a research network that pulls together expertise and resources to tackle some of these applied questions at various levels, through applied research, basic research, sharing of information on surveillance. The funding bodies including government departments and granting agencies, need to be aware of that network as a cohesive entity. There has been a number of partial connections accomplished between individual partners in that potential network, but so far we don't have a network. I think it's somehow we can make that real and meaningful it would be a major step forward. I don't think this exists anywhere else.

- The network concept may fly the best if it is initiated with practical issues in mind. People are seeing this as a more goal-directed thing. Scientists will spontaneously explore…and yet we are here really to accomplish tasks if we can or at least feed into accomplishing tasks. That can be improved if you include in the group people whose job it is to develop policy, to develop regulation, decide where funds go, that deal with decision making on surveillance and testing and so on. They need to be intimately included in the next. It is a relatively novel kind of thing to attempt to undertake goal-directed discussion while trying to include all the best scientists and science. This is highly interdisciplinary and goal-oriented.

- Top of list of priorities is a network…we need to understand the Canadian and American context at the same time. There is consensus that the risk is indistinguishable between the two countries (Mexico's system of meat production may very enough to make their situation different). I would think a North American-wide think tank would be inappropriate thing to create and it should be something that is more than just a one-shot deal where people just get together and agree on a bunch of things and then go away and stop thinking about them. The focus should be on the North American context, and to try to determine first of all what the risks are, and attempt to reach a consensus somehow. People in the regulatory arena and international trade think about these things every day. But those meetings are not particularly scientifically intensive, they tend to be focused on question of surveillance intensity and regulation of the import and export, and so forth. But I think they need to be more scientifically intensive. I think we need to bring in well-chosen international experts who can add their wisdom to the next because they have gone through large experiences already in the UK, and in Europe and Japan. Often in conferences, people do not digest the information on the spot and come up with something for the group and move forward.

- In the future people need to build dedicated facilities if they want to work with prions, and if we want to tackle these issue on a national level, we will have to have national resources with which to do this. We have maybe 20% of what we need in Canada.

- Health Canada and private sector companies do not work together very well. Talented business advisors at Health Canada can help with initial hurdles, but then you run into other issues down the line: who is going to fund it and you have to get all those details worked out in advance or you run into them later at your own detriment. And then companies worry about having people offsite, because of the difficulties with dealing with occupational safety and liability. These things need to be worked out. So, the ability to tap North American potential for companies to come up with solutions to problems out there is greatly underdeveloped.

- The problem of dealing with research in potentially dangerous substances (with prions, and we do not know much about environmental persistence, how much it takes to infected animals were people)…is one of strongest argument for having centralized facilities and resources, perhaps on a cost recovery or profitable basis. The need to have independent research is not an argument for loosening up regulations. Centralized facilities would also help where people don't want to invest in infrastructure, they want to engage in research.

- CFIA laboratory in Lethbridge will be enhanced with additional staff. They are hiring a biologist to work in rapid diagnostic lab and a few technicians as well. They will have more strength in diagnostics. They have thus far not done research, but will do some work with CWD…this has been limited because of the BSE issue. In Alberta as a whole, we have a lot of diagnostic experience—the provincial lab has been doing diagnostics for longtime. But there is not a lot of research experience on the veterinary side and I expect that is true for a lot of Canada. On the human medical side, they have a lot more expertise as far as basic research goes.

- We are relatively new to TSEs. We've had scrapie, but no one in North America paid much attention to it until we had BSE. We have a lot of good international context through the people we have in Canada, but is my impression that we have not taken at far. The UK has a great research program set up, and we are not there yet. The TSEs are a tough area to get into as a young researcher. The scope of what a single project may take is several years because of the incubation period. It takes so long to get anything. A lot of funding agencies will only fund for two years at a time, so if your project goes for five years you might run out of money halfway through. The funding periods may need to be expanded to cover longer periods of time…it often takes six years to get to results in this area.

- The CFIA Winnipeg lab could only handle one or two cows…their animal rooms are quite full. No one seems to know about the Lethbridge facility that has four separate animal rooms that can fit four to five cows in each one…like a large pen with a chute systems. The postmortem room is contained within the facility and it is all a level three facility. So they could have up to 20 adult cow animals at a time. The wing was built to do research in and I believe they are planning to put some elk in that wing this summer and do a pathogenesis study on CWD. So there may be some limitations on using that wing as it will be at least partially filled for the next few years.

Alberta Priorities

- Where to put research funding: live animal testing (diagnostics) would be at top of list. Vaccine development is also important. Find that diagnostics, immunology, and vaccine development all start to happen together. Can always put diagnostics/vaccines as a chunk in the research pile. What happens to the time of exposure to clinical expression is a research target. What happens from the time it goes from gut to brain. We will never be able to do this research in humans. All work will be done in animal models. Other area of research: if there was infected tissue, disposing of it in a way that the risk is minimized and to be able to do this in a large scale. Currently use bleach which is caustic and toxic. The rendering industry: how could you sterilize infected material and put it in a land fill…that would be useful.

- There is a lot of work being done on human prion diseases. The US, Canada, Toronto and in Europe, there is a long ongoing history of prion research in the human context. Anything we do in response to BSE in the human area will probably be duplication. The work needs to be concentrated in BSE and CWD. Maybe we could see how some of the species things differ and use better tools to get there. The study opportunity we have in abundance is CWD.

- Should be looking at having resident expertise in Alberta and if we don't have that, looking at getting it here. It helps to have resident expertise regardless of networks. We have a problem in Canada that we don't have enough specimens to work with, so we might have to go to the UK and work with them. There are benefits to the collaboration and we need to do that.

- In the area of BSE, we are not seen or see ourselves as a leader in this area (but important to be a part of). But we were looking at ourselves in a leadership role in the area of CWD from an animal health and trade restriction, pathogenesis disease transmission and human health perspective. And unique within the environment and it's a western issue and it's an Alberta/Saskatchewan issue.

- VIDO could have set of technologies to help as they are tied into bovine genome project and that will spin off into proteomics research. UK is also keen to work with others (send money).

- In the area of diagnostics, it is just a matter of time before someone finds a much more efficient, less costly and invasive way to diagnose BSE and other prion diseases in animals. Unless we're in it now…I would be less than enthused to see a new diagnostic service developed in Alberta based on current approaches such as of extracting and grinding brains and inserting into mice or using any of the assays used in finding prions.

- To that end I think we need to have someone to push on the development of a diagnostic and provide the resources and the opportunity to move that forward. It depends on the person you recruit whether they would have the ability to capitalize on what is out there on screening tests.

- They have a proposal together (VIDO) to the Canadian Foundation for Innovation (CFI) as lead investor for Level 3 Facility…CFI was a major investor in their Level 2 Facility they just opened. CFI set up for capital funds for research (nanotech centre was one of the funded projects)

- VIDO is not prions-focused as they are generally in the shorter term area and prions need longer term research commitment and there many diseases of higher impact, importance and consequence.

- Should also consider sending some of your scientists to go on work in British labs; works well on the ground level. Maybe send them to the Disease Control Center in Crompton or some of the medical labs in the UK where they're working on this. This doesn't cost an enormous amount of money… build up contacts and the level of knowledge much more rapidly than you can get from just reading journals and articles that are 6 months old. Could stay six months, a year or longer. If there was a prospect of jointly funded collaborative research, this should carry even more weight.

- Science capacity at home… here in Alberta we have some strong research teams in the area of proteomics in general -structural proteomics in particular especially at the University of Alberta and the University of Calgary (and at a tertiary level, the University of Lethbridge). We have people looking at the structure of proteins. Structure, conformation, function, protein protein association, networks, metabolism... all that is central to understanding this disease. When I compare the strengths in Alberta with what exists across the country, certainly there are good protein researchers in Montréal, in Toronto, and I would argue that Alberta is the third strongest in the country in that regard.

- It is clear that if we are going to understand prions and prion related diseases, we need people that are experts in animals physiology, metabolism, heredity (dealing with susceptibility for example), understanding of ruminant biology, feed characteristics and all of that, I would say that we are moderately good in that area... years ago we probably would've been stronger, but here in Alberta like in many places around the world, there has been much less emphasis over the past decades of the core sciences of animal for physiology and metabolism compared with all we had in the 1950s and sixties. We have lost a lot through retirements and people moving on, we have lost much of the strength of what existed here, and that is true of many other places as well. We still have some good people, however, if we were going to mount a concerted effort, to understand prion diseases as it relates to cattle and other ruminants, we would have to do some considerable bolstering. However with, we may find is difficult because many other jurisdictions are coming to the conclusion they have to do work in this area and there will be competition for limited people. If we do identify areas to move forward on and fund, we may find people to attract.

- Our greatest strength is our strong protein research team and secondly, the animal physiology and ruminant biology team. Regarding structural proteomics, in Alberta and linking into Saskatchewan, is the Alberta Synchrotron Institute (www.asi-cls.ca/) and the

Saskatoon based Canadian light source (www.cls.usask.ca/machine/controlinstrumentations.php). These will help us be ideally equipped to determine structural changes and determine prion related protein structure. This allows one to elucidate the structures of proteins and protein complexes with extraordinary detail, and that is important in when it comes to strategies for intervention or advanced diagnostics or almost anything you want to do.

- Now an area I would say we are quite weak, unfortunately, is in the biotechnology, corporate sector. We have virtually no active presence of a major pharmaceutical company in Alberta. There is a lot of activity in the Montréal area, some in Ontario, and virtually nothing in Alberta. We have some small biotech companies. We would have to bring in expertise to bring this to our prairie industries, for cattle, sheep, etc.

5.0 Secondary Information: Literature & Patent Search

Credible published information on BSE and TSE is available in the form of key review documents (from OIE, Harvard University, DEFRA, NIH, etc) and countless articles from scientific journals, along with filed patents. Regarding secondary sources of information, these documents forge to a robust and dynamic overview of the global state of BSE research. Short-listing the over 15,000 articles on TSE and prion research requires a criteria-based approach. Key criterion applied in this analysis included current relevance (i.e., date of publication), experience of researcher (number of publications), and relevance to BSE. Beyond this mechanical approach, researching authors recommended by experts in the field that didn't make the initial short list, were also reviewed for inclusion. Appendix A provides a listing of key scientific articles (with authors and abstracts provided where accessible). Rather than amounting to an end product, this pared down database of key articles presented in the appendices represents a starting point for review and development purposes.

On the foundation of basic science and etiology, commercial activity related to the BSE issue has become intense particularly in the realm of BSE antemortem diagnostics, decontamination. While proprietary issues may create a modest lag on the information available to public research organizations (complicating policy development and resource allocation), filed patents provide a reasonable lead indicator of next generation tools available in the effort to eradicate BSE. An examination of filings over the past year (BSE-related patents) and two years (for prion-related patents) is presented in Appendix B. These patents deal with the following areas of commercial application relevant to BSE: diagnosis and treatment of BSE (and prion-related diseases); transgenic animals (for resistance); sterilization of animal feed, environment, and equipment; and advanced SRM removal techniques.

On August 5, 2002, Dr. Stephen Dealler reported in a website dedicated to prion research (©Priondata.org UK 2002) a relatively detailed perspective on international patents filings. Dealler makes note of the work under the leadership of John Collinge from Imperial College as follows:

- Making beta sheeted PrP artificially for diagnosis or treatment drug screening
- Glycotyping using kits for PrPsc

- Diagnostic using IGEN systems
- Non-aggregated beta PrP for the production of antibodies etc.
- Use of sugar chain changing drugs to treat prion infection.
- Genetic typing and diagnostics using it for susceptibility to TSE.

Prusiner's patents are cited in the following areas:

- Transgenic insertion of a prion protein modulation factor
- Making the prion protein standard
- Use of artificial prion protein complex formation as way of screening compounds for inhibition of the process

Prionics also receives special mention with its work in the following areas:

- Synthetic polypeptide interacting with anti-PrPsc
- Specific peptides to create or interact with anti-PrP
- With Electronic Microtech in Switzerland the use of specific anti-PrPsc as a diagnostic tool. Electrophoretic separation of the prion proteins
- Glycotyping using kits for PrPsc
- Diagnostic using IGEN systems
- Western blotting looking for a 35-39kDa protein in CSF
- Separation of PrP from tissues

In the realm of treatments, the following areas of patent filings are noted:

- Use of sugar chain changing drugs to treat prion infection.
- Use of suspensions of amyloidal proteins to screen potential treatments.
- Use of artificial prion protein complex formation as way of screening compounds for inhibition of the process.
- Leucodepletion to remove infection from blood.
- Polyionised saccharides as a potential treatment for amyloidal disease.
- Compounds active against apoptosis
- Mechanisms to prevent the formation of discordant helices
- Compounds acting against endoplasmic reticulum damage

The assumption that commercial potential for a BSE diagnostic exceeds that of other areas of commercial develop appears valid given the vast number of related patents that have been filed:

- The extraction of PrPsc from mononuclear cells
- Western blotting looking for a 35-39kDa protein in CSF
- PrPsc specific antibodies (NB Prusiner claims to cover this in a previous patent but it is unclear how specific his patent is)
- The use of guanidine thiocyanate to increase the signal to noise ratio in diagnostics antibody based kits
- PrPsc in meat as an indication of infectivity (and the lack of it as an indication of no infectivity)
- Separation of PrP from tissues
- High affinity monoclonal for diagnostics (but not specific for PrPsc)
- Making the prion protein standard
- Synthetic polypeptide interacting with anti-PrPsc
- PrP interaction with short peptide
- Overexpression of proteins in TSE infections: Laminin Receptor/precursor and gamma interferon and heart and brain fatty acid proteins
- The Delfia immunoassay for PrP
- Acinetobacter antibodies as an indicator of prion disease
- Prionins as diagnostic factors in prion disease. The proof of the method is unclear.
- Plasminogen as a molecule that will specifically interact with PrPsc (repeat 1,
- Aptamers instead of antibodies for specific interaction with PrP (repeat 1 against PrPsc)
- With Electronic Microtech in Switzerland the use of specific anti-PrPsc as a diagnostic tool.
- This is the optical equivalent of the patent above
- Confocal microscopy of fluorescent antibody labeled PrPsc inside cells.PrPsc specific ligands for use in diagnostics, and ones using small peptides.
- Specific anti-PrPsc and the peptide used to make it.

- Prion protein binding proteins initially felt to be useful in separation of PrPc from PrPsc.Prion related protein that is in some way useful in diagnostics (it is in German) (repeat 1, repeat 2)
- Prion-like polynucleotides/polypeptides
- DNA-PrP linkages
- Measurement of respiratory sinus arrythmia changes
- Sets of antibodies
- Specific antiPrPsc monoclonal from Japan
- Another immunoassay for PrP
- Urine test for proteinase resistant PrP
- Polyclonals specifically active against PrPsc
- Peptide ligands for PrP 1, 2.
- Soto's method for seeding prions and multiplication using ultrasound
- Copolymers (i.e. polymers of 2 positively charged aminoacids) that adhere to PrP
- Testing for infection in meat by looking for PrPsc elsewhere and a specific genetic fingerprint
- Immuno-PCR for PrPsc

In the area of transgenics, the following patents were noted:

- Transgenic mice found using specific methods (NB most of the patents on transgenics are under Prusiners' group)
- Transgenic insertion of a prion protein modulation factor
- Ungulates not carrying the prion protein
- A hypohalite
- Sequestrants to take out the prions
- Protein hydrolysis
- Biological indicators of prion destruction. i.e. items that are destroyed in a similar manner

Appendix A

Database of Select Research Publications and Abstracts – By Author

Database of Select Research Publications and Abstracts – By Author

1. Aguzzi, A. (2001). From Cattle to Humans: Which Connections? Biofutur, 2001, 26-29.

2. Aguzzi, A., & Brandner, S. (1999). The genetics of prions - a contradiction in terms? Lancet, 354(Suppl. 1), SI22-SI25.

3. Andrews, N. J., Farrington, C. P., Cousens, S. N., Smith, P. G., Ward, H., Knight, R. S. G., Ironside, J. W., & Will, R. G. (2000). Incidence of Variant Creutzfeldt-Jakob Disease in the Uk. Lancet, 356(9228), 481-482.

   Abstract: The number of deaths from variant CJD (vCJD) in the UK increased in the last quarter of 1998, although numbers were lower in subsequent quarters. We analysed the numbers of definite and probable (living and dead) vCJD cases since 1994 to assess trends in incidence. We estimated that the number of onsets increased by 23% per year for 1994-2000 (p=0.004), and that deaths increased by 33% for 1995-2000 (p=0.005). The absolute number of cases in the UK is still low, but such an increase should be a matter of concern.

4. Andrews, N. J., Farrington, C. P., Ward, H. J. T., Cousens, S. N., Smith, P. G., Molesworth, A. M., Knight, R. S. G., Ironside, J. W., & Will, R. G. (. Deaths from variant *Creutzfeldt*-Jakob disease in the UK. LANCET, 361 (9359): 751-752 MAR 1 2003.

   Abstract: In 2002, 17 people died. from variant *CJD* (*vCJD*) in the UK, compared with 20 in 2001 and 28 in 2000. We analysed data for deaths from *vCJD* since 1995 and estimated the underlying trend in mortality. The trend had a quadratic component (p=0.005), suggesting that the Increase was not exponential, and that the previously increasing trend is slowing down. The death rate peaked in 2000. These findings are encouraging, but mortality might increase again in the future. DOCUMENT TYPE: Article

5. Appel, T. R., Wolff, M., Von Rheinbaben, F., Heinzel, M., & Riesner, D. (2001). Heat Stability of Prion Rods and Recombinant Prion Protein in Water, Lipid and Lipid-Water Mixtures. Journal of General Virology, 82, 465-473.

   Abstract: Prion rods, i.e. insoluble infectious aggregates of the N-terminally truncated form of the prion protein, PrP 27-30, and the corresponding recombinant protein, rPrP(90-231), were autoclaved in water, bovine lipid or lipid-water mixtures for 20 min at temperatures from 100 to 170 degreesC. A protocol was developed for the quantitative precipitation of small amounts of protein from large excesses of lipid. PrP remaining undegraded after autoclaving was quantified by Western blot and degradation factors were calculated. The Arrhenius plot of the rate of degradation vs temperature yielded linear relationships for prion rods in water or lipid-water as well as for rPrP(90-231) in lipid-water. The presence of lipids increased the heat stability of prion rods, especially at lower temperatures. Prion rods had a much higher thermal stability compared to rPrP, Autoclaving of prion rods in pure lipid gave different results - not simple degradation but bands indicative of covalently linked dimers, tetramers and higher aggregates. The heat stability of prion rods in pure lipid exceeded that in lipid-water mixtures. Degradation factors larger than 10(4) were reached at 170 degreesC in the presence of lipids and at 150 degreesC in the absence of lipids. The linear correlation of the data allows cautious extrapolation to conditions not tested, i.e. temperatures higher than 170 degreesC. A factual basis for assessing the biological safety of industrial processes utilizing potentially BSE- or scrapie-contaminated animal fat is provided.

6. Barnard, G., Helmick, B., Madden, S., Gilbourne, C., & Patel, R. (2000). The Measurement of Prion Protein in Bovine Brain Tissue Using Differential Extraction and Delfia (R) as a Diagnostic Test for Bse. Luminescence, 15(6), 357-362.

   Abstract: A simple diagnostic test for the detection of bovine spongiform encephalopathy (BSE), based on a commercially available time-resolved fluorescence immunoassay (DELFIA(R)) for the measurement of the normal and disease-associated isoforms of prion protein (PrP), is described. The isoforms are sequentially extracted from homogenized bovine brain tissue using two concentrations of guanidine hydrochloride. This procedure initially extracts a soluble isoform and subsequently a less soluble disease-associated aggregated isoform. Following quantification of the two fractions, the percentage of the insoluble prion becomes a measurable parameter, independent of protein concentration, clearly identifying normal from infected animals displaying clinical signs of BSE. The mean percentages of insoluble PrP in brain tissue from 60 BSE-confirmed-positive cattle and 100 cattle that had never been exposed to the disease were 52.6% (SD = 22.8) and 3.9% (SD = 1.5), respectively. The assay is sensitive, with a detection limit of less than 50 pg PrP, and is robust and precise (CVs < 10%) over the appropriate working range. Copyright (C) 2000 John Wiley & Sons, Ltd.

7. Baron, T. G. M., & Biacabe, A. G. (2001). Molecular Analysis of the Abnormal Prion Protein During Coinfection of Mice by Bovine Spongiform Encephalopathy and a Scrapie Agent. Journal of Virology, 75(1), 107-114.
   Abstract: Molecular features of the proteinase K-resistant prion protein (PrP res) may discriminate among prion strains, and a specific signature could be found during infection by the infectious agent causing bovine spongiform encephalopathy (BSE), To investigate the molecular basis of BSE adaptation and selection, we established a model of coinfection of mice by both BSE and a sheep scrapie strain (C506M3), We now show that the PrP res features in these mice, characterized by glycoform ratios and electrophoretic mobilities, may be undistinguishable from those found in mice infected with scrapie only, including when mice were inoculated by bath strains at the same time and by the same intracerebral inoculation route. Western blot analysis using different antibodies against sequences near the putative N-terminal end of PrP res also demonstrated differences in the main proteinase K cleavage sites between mice showing either the BSE or scrapie PrP res profile. These results, which may be linked to higher levels of PrP res associated with infection by scrapie, were similar following a challenge by a higher dose of the BSE agent during coinfection by both strains intracerebrally. Whereas PrP res extraction methods used allowed us to distinguish type 1 and type 2 PrP res, differing, like BSE and scrapie, by their electrophoretic mobilities, in the same brain region of some patients with Creutzfeldt-Jakob disease, analysis of in vitro mixtures of BSE and scrapie brain homogenates did not allow us to distinguish BSE and scrapie PrP res. These results suggest that the BSE agent, the origin of which remains unknown so far but which may have arisen from a sheep scrapie agent, may be hidden by a scrapie strain during attempts to identify it by molecular studies and following transmission of the disease in mice.

8. Bastian, F. O.//Foster, J. W. (2001 Jun). Spiroplasma Sp. 16s Rdna in Creutzfeldt-Jakob Disease and Scrapie as Shown by Pcr and Dna Sequence Analysis. Journal of Neuropathology and Experimental Neurology
   Abstract: The pathogenesis of the transmissible spongiform encephalopathies (TSE), which include Creutzfeldt-Jakob disease (CJD) in humans and scrapie in sheep. remains an enigma. In this paper we present evidence for the association of Spiroplasma sp., a wall-less prokaryote. with TSE. We have shown PCR amplification or Spiroplasma 16S rDNA in TSE-infected brain tissues (13 of 13 CJD cases and 5 of 9 scrapie cases) and not in control brains (0 of 50). Direct sequencing of the amplified PCR products has confirmed the presence of Spiroplasma-like DNA in all 5 of the TSE brains tested. Our evidence is nor necessarily in conflict with involvement of a PrPres-a protease-resistant host-derived protein referred to as the prion-in the pathogenesis of TSE. since there is evidence that another factor is involved. We propose a bacterium. namely Spiroplasma. as this associated factor although the role of Spiroplasma in TSE cannot be determined from these experiments. The presence of the nucleic acid sequence of this microbe in all cases of TSE in our laboratory and not in controls provides direct evidence of the association of Spiroplasma sp. with TSE.

9. Belay, E. D., Maddox, R. A., Gambetti, P., & Schonberger, L. B. (2003). Monitoring the occurrence of emerging forms of Creutzfeldt-Jakob disease in the United States. Vol. 60, 176-181.
   Abstract: Transmissible spongiform encephalopathies (TSEs) attracted increased attention in the mid-1980s because of the emergence among UK cattle of bovine spongiform encephalopathy (BSE), which has been shown to be transmitted to humans, causing a variant form of Creutzfeldt-Jakob

disease (vCJD). The BSE outbreak has been reported in 19 European countries, Isreal, and Japan, and human cases have so far been identified in four European countries, and more recently in a Canadian resident and a US resident who each lived in Britain during the BSE outbreak. To monitor the occurence of emerging forms of CJD, such as vCJD, in the United States, the Centers for Disease Control and Prevention has been conducting surveillance for human TSEs through several mechanisms, including the establishment of the National Prion Disease Pathology Surveillance Center. Physicians are encouraged to maintain a high index of suspicion for vCJD and use the free services of the pathology center to assess the neuropathology of clinically diagnosed and suspected cases of CJD or other TSEs.

10. Betemps, D., & Baron, T. (2001). Molecular Specificities of Antibodies Against Ovine and Murine Recombinant Prion Proteins. Biochemical and Biophysical Research Communications, 281(1), 101-108.
Abstract: The prion proteins (PrP) from sheep and mouse were produced in large quantities of full-length protein in Escherichia coli after fusion with a carboxyterminal hexahistidine sequence. Both recombinant proteins were recognized, at variable levels, in ELISA using a panel of antibodies recognizing different parts of the PrP molecules, from the octo-repeat region (79-92 human sequence), to the C terminal end of the protein. We show that these recombinant proteins enable polyclonal antisera to be produced in PrP0/0 mice, the sheep prion protein being strongly immunogenic, using either native or guanidium hydrochloride-treated recombinant protein. Sera produced against the sheep protein also reacted in Western blot with bovine, ovine, and murine PrP res, but showed higher reactivity with sheep PrP res. interestingly, when compared to an antiserum produced against bovine 106-121 peptidic sequence (RB1), we found strikingly different ratios of the PrP res glycoforms, in both cattle with BSE and sheep with natural scrapie, but not in scrapie infected mice. Such results further demonstrate that the assessment of PrP res glycoform ratios, using different antibodies, may depend on antibodies species-specificities. (C) 2001 Academic Press.

11. Bosque, P. (2002). Bovine spongiform encephalopathy, chronic wasting disease, scrapie, and the threat to humans from prion disease epizootics. Current Neurology and Neuroscience Reports, 2(6), 488-95.
Abstract: Ongoing endemics and epidemics of prion disease afflict several species or ruminants regularly consumed by humans. Bovine spongiform encephalopathy (BSE) is epidemic in British cattle, and is now found in the cattle of more than 20 countries. A large, and apparetnly growing epidemic of chronic wasting disease plagues deer and elk in North America. Finally, scrapie has been endemic int he sheep of most coutnries for many decades. It was once assumed that humans were not susceptible to these ruminant forms of prion idisease, but an outbreak of a new form of Creutzfeld-Jakob disases (CJD) among young Britons, almost certainly due to dietary exposure to BSE-contaminated beef, has disproved this supposition. Although all prion diseases share the same fundamental pathologic mechanism, transmission between species is sometimes inefficient. The basis of this 'species barrier' is incompletely understood, but interspecies differences in the amino acid sequence of the prion protein and the strain of prions involved play criticl roles. Reliable experimental models for dteermining the resistance fo humans to animal prion diseases do not yet exist. It is possible that animal to human transmission of prion disease may manifest as CJD with unusual characteristics. but this is not necessarily the case. In the absence of a reliable means for determining the susceptibility of humans to animal prio disases, measures to minimize human exposure to animal prions should be emphaisized.

12. Brown DA, Bruce ME, & Fraser JR. (2003). Comparison of the neuropathological characteristics of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD) in mice. NEUROPATHOLOGY-AND-APPLIED-NEUROBIOLOGY. JUN 2003; 29 (3) : 262-272.
Abstract: Bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD) belong to a group of diseases called the transmissible spongiform encephalopathies (TSEs). Transmission studies in inbred mice (strain typing) provided overwhelming evidence that vCJD arose from BSE. In this study, we compare the patterns of neuropathology in a panel of three inbred mouse strains (RIII, C57BL and VM) and one cross (C57BL x VM) infected with either vCJD or BSE. For each mouse strain, patterns of abnormal prion protein (PrPres) deposition, astrocytosis and vacuolation were similar in the vCJD- and BSE-challenged mice. Prion protein (PrP)-positive

plaques were prominent in the VM and C57BL x VM mice in addition to diffuse PrPres accumulation, whereas only diffuse PrPres labelling was observed in the RIII and C57BL mice. The hippocampus was targeted in all mouse strains, as was the cochlear nucleus in the medulla, both showing consistent severe vacuolation and heavy PrPres deposition. Although the targeting of PrPres was similar in the BSE- and vCJD-infected brains, the amount and intensity of PrPres observed in the brains treated with formic acid during fixation was reduced considerably. The distribution of astrocytosis was similar to the targeting of PrPres deposition in the brain, although some differences were observed in the hippocampi of mice challenged with vCJD. We conclude that there are no significant differences in the targeting of neuropathological changes observed in the BSE- and vCJD-infected mice, consistent with the previous evidence of a link between BSE and vCJD.

13. Brown, P., Will, R. G., Bradley, R., Asher, D. M., & Detwiler, L. (2001). Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease: Background, Evolution, and Current Concerns. Emerging Infectious Diseases, 7(1), 6-16.
Abstract: The epidemic of bovine spongiform encephalopathy (BSE) in the United Kingdom, which began in 1986 and has affected nearly 200,000 cattle, is waning to a conclusion, but leaves in its wake an outbreak of human Creutzfeldt-Jakob disease, most probably resulting from the consumption of beef products contaminated by central nervous system tissue. Although averaging only 10-15 cases a year since its first appearance in 1994, its future magnitude and geographic distribution (in countries that have imported infected British cattle or cattle products, or have endogenous BSE) cannot yet be predicted. The possibility that large numbers of apparently healthy persons might be incubating the disease raises concerns about iatrogenic transmissions through instrumentation (surgery and medical diagnostic procedures) and blood and organ donations. Government agencies in many countries continue to implement new measures to minimize this risk.

14. Brown, P., Meyer, R., Cardone, F., & Pocchiari, M. (2003). Ultra-high pressure inactivation of prion infectivity in processed meat: A practical method to prevent human infection. Proc Natl Acad Sci U S A, 100(10), 6093-7.
Abstract: Bovine spongiform encephalopathy contamination of the human food chain most likely resulted from nervous system tissue in mechanically recovered meat used in the manufacture of processed meats. We spiked hot dogs with 263K hamster-adapted scrapie brain (10% wt/wt) to produce an infectivity level of about 9 log10 mean lethal doses (LD50) per g of paste homogenate. Aliquots were subjected to short pressure pulses of 690, 1,000 and 1,200 MPa at running temperatures of 121-137 degrees C. Western blots of PrPres were found to be useful indicators of infectivity levels, which at all tested pressures were significantly reduced as compared with untreated controls: from about 10^3 LD50 per g at 690 MPa to about 10^6 LD50 per g at 1,200 MPa. The application of commerically practical conditions of temperature and pressure could ensure the safety of processed meats from bovine spongiform encephalopahty contamination, and could also be sused to study phase transitions of the prion protein from its normal to misfolded state.

15. Bruce ME. (2003). TSE strain variation. BRITISH-MEDICAL-BULLETIN. 2003; 66 : 99-108.
Abstract: Studies in mice have revealed considerable strain variation in the agents causing transmissible spongiform encephalopathies (TSEs). TSE strains interact with genetic factors in the host (in particular PrP genotype) to influence characteristics of the disease such as incubation period and neuropathology. TSE strains can retain their identity after propagation in different host species or PrP genotypes, showing that these agents carry their own strain-specific information. It is not known whether this information resides in specific self-perpetuating modifications of PrP, or whether a separate informational molecule is required. Strain typing in mice can be used to explore links between TSEs occurring naturally in different species. Such studies have demonstrated that the strain causing BSE in cattle has also infected domestic cats and exotic ungulates. Most importantly, the BSE strain has also been isolated from patients with variant CJD. In contrast, different TSE strains are associated with sporadic CJD and sheep scrapie.

16. Campbell, S., Dennehy, U., & Telling, G. (2000). Analyzing the Influence of Prp Primary Structure on Prion Pathogenesis in Transgenic Mice. Archives of Virology, 87-94.
Abstract: Expression of prion protein (PrP) genes in transgenic (Tg) mice has been an extremely

effective means of studying human and animal prion diseases. Indeed, much of what we currently understand about the molecular basis of prion pathogenesis derives from such studies. Despite these advances, the emergence of a new variant of Creutzfeldt-Jakob disease (vCJD), apparently the human manifestation of bovine spongiform encephalopathy (BSE), demonstrates that our understanding of the factors controlling prion transmission is far from complete. We review studies in Tg mice that have addressed issues of prion strains and species barriers and have provided insights into mechanisms of prion propagation. The goal of future investigation will be to determine the interplay between PrP primary structure and conformation in determining prion transmission barriers and we discuss some ongoing transgenic studies designed to address these issues.

17. Carson, C., Mckay, J. S., Brooks, H. W., Kelly, D. F., Stidworthy, M. F., Wibbelt, G., & Morgan, K. L. (2001). Establishment and Maintenance of a Longitudinal Study of Bovine Spongiform Encephalopathy (the Ulises Scheme). Preventive Veterinary Medicine, 51(3-4), 245-257.
Abstract: This paper addresses the issues of tracing and compliance encountered in setting up and maintaining a UK-wide 5-year observational study of beef cattle. The 5-year prospective study was initiated in 1997 to investigate the occurrence of bovine spongiform encephalopathy (BSE) in a single herd of pedigree Aberdeen Angus cattle, in which BSE had been detected at low prevalence. The study was given the acronym ULiSES (University of Liverpool Spongiform Encephalopathy Scheme). All cattle present on the farm at the start of the scheme were registered as members of the study population (n = 320), as were all calves subsequently born on the farm (n = 350). Animals that were sold (n = 291) were traced and monitored at destination farms. Farmers were requested to give advance notification of slaughter of any ULiSES animal and an attempt was made to collect post-mortem samples of nervous tissue, peripheral lymphoid tissue and striated muscle from all animals in the scheme at the time of slaughter, death or euthanasia. Sections of medulla were examined (by standard histopathological techniques) for the presence of spongiform change. Remaining samples were stored at -70 degreesC for future investigation by alternative tests. At the halfway point of the scheme in October 1999, 75.2% (506/673) of the study population was still alive; 42% (284) of the population was still alive on the study farm and 33% (222) was distributed on other farms throughout the UK. Complete set,, or specimen,, had been recovered from 77% (129/167) of dead animals. All brainstem sections were negative by histopathological examination. No suspect cases of BSE were reported in ULiSES animals. Failure to recover specimens occurred principally in animals which had left the study farm. The main cause of specimen loss was a failure of compliance in a small number of individuals who had them without purchased large numbers of ULiSES animals, and subsequently slaughtered them without contacting the University. Despite this. farmer compliance was generally high. The ULiSES scheme shows the feasibility of a country-wide longitudinal observational study spanning a period of several years and indicates the large impact of small numbers of non-compliant individuals. (C) 2001 Elsevier Science B.V. All rights reserved.

18. Cashman, N. (1999). A cell surface receptor for the prion protein. Transmissible Sponigform Encephalopathies. Cambridge Healthtech Institute. October 27-28. Wahsington DC .

19. Cashman, N. R. (2001). Transmissible spongiform encephalopathies: vaccine issues. Dev Biol (Basel), 106, 455-9; discussion 460-1, 465-75.
Abstract: The recent emergence of bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob Disease (vCJD) suggests that transmissible spongiform encephalopathies (TSEs) pose an ongoing threat to human and animal health. To avoid iatrogenic transmission of TSEs in vaccines, strategies must be developed to obviate TSE agent infectivity in cellular substrates, cell culture media components and enzymes, and excipients, and to validate the safety of these components and field vaccines efficiently

20. Castilla, J., Gutierrez Adan, A., Brun, A., Pintado, B., Ramirez, M. A., Parra, B., Doyle, D., Rogers, M., Salguero, F. J., Sanchez, C., Sanchez-Vizcaino, J. M., & Torres, J. M. (2003). Early detection of PrPres in BSE-infected bovine PrP transgenic mice. Arch Virol, 148(4), 677-91.
Abstract: Transgenic mouse lines expressing different levels of the bovine prion protein gene (boPrP(C)) were generated. Upon infection with BSE prions, all transgenic lines tested exhibited characteristics of the bovine disease. Typical CNS spongiform degeneration was observed by

histopathology and presence of PrP(res) could be detected both by Western blot and immunohistochemistry (IHC) assays, confirming for this model the absence of an interspecies barrier to BSE infection. Differences in incubation times post-inoculation depend upon the expression level of boPrP(C) and the amount of prions in the inoculum. In the absence of clinical signs, pathognomonic markers of disease could be detected as early as 150 or 196 days post-inoculation by IHC and Western blot analysis, respectively. This result indicates that prion infectivity in experimental mouse bioassays can be measured earlier by assessing immunologically the presence of PrP(res) in brains from inoculated animals. Although these transgenic mice were also susceptible to sheep scrapie prion infection, the extent of incubation times was considerably longer and PrP(res) was detected in only 70 % of inoculated mice. Interestingly, transgenic mice-propagated sheep scrapie prions displayed distinct biochemical properties when compared to both the original sheep scrapie and transgenic mouse-propagated BSE inoculum.

21. Caughey B. (2003). Probing for prions: recognizing misfolded PrP. NATURE-MEDICINE. JUL 2003; 9 (7) : 819-820.
Abstract: New antibodies specific for the misfolded, and presumably pathogenic, form of the PrP protein have now made their long-awaited debut. These antibodies could provide tools for diagnostics, research and therapy (pages 893-899).

22. Cohen, C. H. (2000). Does Improvement in Case Ascertainment Explain the Increase in Sporadic Creutzfeldt-Jakob Disease Since 1970 in the United Kingdom? American Journal of Epidemiology, 152(5), 474-479.
Abstract: The aim of this study was to quantify the improvements in case ascertainment which are considered to explain the rise in the incidence of sporadic Creutzfeldt-Jakob disease. The numbers of cases, falling into five 10-year age groups starting at the age of 30 and three calendar periods of report since 1970, were analyzed by Poisson regression, assuming a constant age distribution. The age-period and age-cohort models were applied and discussed. The age-period model showed that underreporting in 1970-1979 was greater among patients aged 70 years or older. The age-cohort model indicated that a cohort factor increased over the first half of the 20th century (e.g., the incidence in the generation born in 1940 was almost twice that in the generation born in 1920); this increase was probably an artifact due to the past underascertainment pattern. However, from a statistical viewpoint, both models lead to a good fit; the cohort factor may appear to be as relevant as the period factor in describing the trends in incidence. Thus, one can imagine an unlikely worst case scenario, assuming that an unknown cohort factor is involved, in that case, the age-cohort model gives more optimistic predictions than Neilson's model (BMJ 1996;312:1038-9). These results are consistent with both interpretations: The rise in incidence is governed by improvements in case ascertainment, and is greater among old people (the most accepted interpretation); this rise may depend on a cohort factor as well, which may correspond to the zoonotic hypothesis (a totally hypothetical interpretation). Interpreting the increase of sporadic Creutzfeldt-Jakob disease over generations in terms of exposure to putative environmental factors is still a matter of debate; ongoing epidemiologic surveys may provide more information. Presently, this increase can be explained as an artifact due to the past underreporting pattern, with 79% (95% confidence interval: 56, 90) of the cases among persons aged greater than or equal to 70 years being missed in 1970-1979.

23. Cohen, F. E. (2000). Prions, Peptides and Protein Misfolding. Molecular Medicine Today, 6(7), 292-293.

24. Cohen FE, & Kelly JW. (2003). Therapeutic approaches to protein-misfolding diseases. NATURE-. DEC 18 2003; 426 (6968) : 905-909.
Abstract: Several sporadic and genetic diseases are caused by protein misfolding. These include cystic fibrosis and other devastating diseases of childhood as well as Alzheimer's, Parkinson's and other debilitating maladies of the elderly. A unified view of the molecular and cellular pathogenesis of these conditions has led to the search for chemical chaperones that can slow, arrest or revert disease progression. Molecules are now emerging that link our biophysical insights with our therapeutic aspirations.

25. Collinge, J. (1997). Human prion diseases and bovine spongiform encephalopathy (BSE). Hum Mol Genet,

6(10), 1699-705.
Abstract: Prion diseases are transmissible neurodegenerative disorders which affect a range of mammalian species. In humans they can be inherited and sporadic as well as acquired by exposure to human prions. Prions appear to be composed principally of a conformational isomer of host-encoded prion protein and propagate by recruitment of cellular prion protein. Recent evidence argues that prion protein can also encode disease phenotypes by differences in its conformation and glycosylation. Such molecular analysis of prion strains suggests that new variant Creutzfeldt-Jakob disease is caused by BSE exposure. The novel biology of prion propagation may not be unique to these rare degenerative brain diseases.

26. Collinge, J. (2001). Prion Diseases of Humans and Animals: Their Causes and Molecular Basis. Annual Review of Neuroscience, 24, 519-550.
Abstract: Prion diseases are transmissible neurodegenerative conditions that include Creutzfeldt-Jakob disease (CJD) in humans and bovine spongiform encephalopathy (BSE) and scrapie in animals. Prions appear to be composed principally or entirely of abnormal isoforms of a host-encoded glycoprotein, prion protein. Prion propagation involves recruitment of host cellular prion protein, composed primarily of alpha -helical structure, into a disease specific isoform rich in beta - sheet structure. The existence of multiple prion strains has been difficult to explain in terms of a protein-only infections agent, but recent studies suggest that strain specific phenotypes can be encoded by different prion protein conformations and glycosylation patterns. The ability of a protein to encode phenotypic information has important biological implications. The appearance of a novel human prion disease, variant CJD, and the clear experimental evidence that it is caused by exposure to BSE has highlighted the need to understand the molecular basis of prion propagation, pathogenesis, and the barriers limiting inter-mammalian transmission. It is unclear if a large epidemic of variant CJD will occur in the years ahead.

27. Cooper, J. D., & Bird, S. M. (2001). Analysis of the Bovine Spongiform Encephalopathy Maternal Cohort Study, Revisited. Journal of the Royal Statistical Society Series C-Applied Statistics, 50, 313-324.
Abstract: The bovine spongiform encephalopathy (BSE) maternal cohort study provided robust evidence of an enhanced risk of developing BSE for offspring of BSE-affected dams. We present for the first time, but in retrospect, an interim analysis of the BSE maternal cohort study and set it in historical context, some of which has only been revealed through the BSE inquiry. We also consider the implications for design of extending the BSE maternal cohort study once an enhanced risk to exposed calves had been established, to assess the risk to calves born further from the clinical onset of BSE in the dam than those in the original study. We demonstrate that, if a data monitoring committee had been established, conclusions similar to those based on the final results could have been drawn several years before the completion of the BSE maternal cohort study. Further, we conclude that an extension of the cohort study is unlikely to have been commissioned because of the substantial financial investment required, yet low power, and practical difficulties associated with implementation of any worthwhile extension.

28. Coulthart, M. B., & Cashman, N. R. (2001). Variant Creutzfeldt-Jakob Disease: a Summary of Current Scientific Knowledge in Relation to Public Health. Canadian Medical Association Journal, 165(1), 51-58.
Abstract: The prion diseases pose unique scientific, medical, veterinary and regulatory challenges. Here, we summarize current information bearing on the natural history, pathobiology and epidemiology of these disorders and public policy responses to the potential threats to public health posed, particularly, by bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease (vCJD). Six years after the first case reports of vCJD, there is still no clear indication of the magnitude of the primary epidemic, or of the likelihood of lateral transmission of this untreatable disease by iatrogenic means, particularly by blood and blood products. However, the unsettling nature of the available evidence warrants prudence regarding public health policy and regulation, as well as a forward-looking approach to research.

29. Dagvadorj, A., Petersen, R. B., Lee, H. S., Cervenakova, L., Shatunov, A., Budka, H., Brown, P., Gambetti, P., & Goldfarb, L. G. (. Spontaneous mutations in the *prion* *protein* gene causing

transmissible *spongiform* *encephalopathy*. ANNALS OF NEUROLOGY, 52 (3): 355-359 SEP 2002.

Abstract: We analyzed the *prion* *protein* gene (PRNP) region in patients with *transmissible* *spongiform* *encephalopathy* associated with the PRNP D178N mutation. The results suggest that the D178N chromosomes had independent origins in each affected pedigree or apparently sporadic case. A de novo spontaneous PRNP mutation was observed. We provide evidence that hereditary and apparently sporadic *transmissible* *spongiform* *encephalopathy* cases associated with the D178N mutation result from multiple recurrent mutational events. DOCUMENT TYPE: Article

30. Daniel, H. (2001). From Protein Folding to Bse Crisis: Present Knowledge of Structure and Function of Prion Proteins. Ernahrungs-Umschau, 48(2), 48-+.

    Abstract: The pathogenesis of prion diseases is based on a novel mechanism by which a cellular protein (PrPc) obtains a new conformation (PrPSc) that in turn renders in a self-replicating manner other normal prion proteins into the pathogenic form. This new conformer is characterized by a decreased content of helical structures and an increased fraction of beta-sheet like structures. The protein thereby gains a high resistance to all kinds of denaturating agents as well as against most proteases. This allows the protein to be transmitted along a food chain and permits its entry into the body in intact form via the gastrointestinal tract of most mammals. Although its replication within an organism requires the presence of normal prion proteins, there is a number of not yet well characterized factors that either promote and/or prevent formation of the pathogenic prion forms and therefore determine the susceptibility of an individual for the disease. Prion diseases may be considered as protein folding diseases and can be acquired a) as a sporadic form by spontaneous mutations b) in an inherited autosomal-dominant form or c) by alimentary intake via foods containing,infectious' prions such as those causing BSE in cattle.

31. Dear, D. V., Fitzmaurice, T. J., Garton, S., & Richards, S. J. (2001). Pilot Study to Determine the Feasibility of Producing Protease-Resistant Prion Protein Fragments by Random Pcr Mutagenesis. Biochemical and Biophysical Research Communications, 281(4), 929-935.

    Abstract: We report the results of a pilot study to determine the feasibility of using PCR random mutagenesis and in vitro transcription/translation to produce protease resistant full-length or truncated ovine prion proteins (PrP). Using this approach, we show the novel production of protease resistant recombinant ovine prion protein fragments isolated from a panel of seventy randomly mutated ovine PrP protein molecules. Protease resistance of the proteinase K (PK) digested fragments was present de novo within physiological conditions without the need for template-assisted conversion to protease resistance or the requirement of reductants, denaturants or acid pH reported to date. Four of the mutant proteins were truncated at their C-termini and all of these gave rise to digestion products which were protease resistant at significant PK concentrations and exposure times. All other mutant proteins translated as full length molecules and gave rise to PK-resistant products which showed a variability in their proteinase digestion profiles. We discuss the relevance of these finding to current research. (C) 2001 Academic Press.

32. DeArmond, S. J., & Bouzamondo, E. (. Fundamentals of *prion* biology and *diseases*. TOXICOLOGY, 181: 9-16 DEC 27 2002.

    Abstract: One of the most remarkable changes in medicine during the last 20 years of the 20th century was the shift from the clinical-neuropathological classification of *Creutzfeldt*-Jakob *disease* (*CJD*) and related disorders as '*transmissible* *spongiform* *encephalopathies*' to a molecular-etiologic classification as '*prion* *diseases*'. We now know that these *diseases* are caused by abnormalities of the *prion* *protein* (PrP). Specifically, *CJD* is caused by the conversion of the normal, protease-sensitive PrP isoform, designated PrPcC, to a protease resistant isoform, designated PrPSc. PrPSc forms into an infectious particle, named a '*prion*', that can transmit the *disease*. Accumulation of PrPSc in the brain causes neurodegeneration. The main goals of this review are to summarize our understanding of the attributes of the PrP molecule that give it the properties of an infectious agent and to describe how different alterations of the PrP molecule cause the multiple known *prion* *disease* variants. Finally, the emergence of a new variant of *CJD* in Great Britain and to a lesser extent in Europe and its relationship to the emergence of a particularly virulent form of *bovine* *spongiform* *encephalopathy* will be discussed. (C) 2002 Published by Elsevier Science Ireland Ltd. DOCUMENT TYPE: Article

33. Deslys, J. P., Lasmezas, C. I., Comoy, E., & Domont, D. (2001). Diagnosis of Bovine Spongiform Encephalopathy. Veterinary Journal, 161(1), 1-3.

34. Deslys JP. (2003). Prions and risks for blood transfusion: the situation in 2003. TRANSFUSION-CLINIQUE-ET-BIOLOGIQUE. JUN 2003; 10 (3) : 113-125.
    Abstract: In 2003, Prions still constitute a biological enigma and a public health concern. The risks of transmission of the so called 'mad cow disease' are now under control but concerns still persist about potential secondary transmissions, notably via blood transfusion. Information obtained from diseases previously observed in animals (scrapie of sheep and goat) and in man (Kuru, Creutzfeldt-Jakob disease) demonstrate the complexity of the relations between these transmissible agents and their host. The difficulty in decontamination, the very long silent incubation period during which diagnosis is not possible and the lack of treatment are alarming elements which explain the increased perception of risk for these diseases. The development of rapid screening tests used on bovine at slaughterhouse has represented an important improvement in the development of a targeted protection against these agents. Today, technical evolutions in diagnosis let us imagine the possibility of blood detection for prions: on one hand new garanties for security may arise but on the other hand it points out the potential infectivity of blood with these agents responsible for constant fatal diseases. Precautionary security measures have to ensure an optimal ratio benefit/risk for the patient and thus, in this field, to balance the risk linked to prions with those clearly identified elsewhere. (C) 2003 Publie par Editions scientifiques et medicales Elsevier SAS.

35. Dormont, D. (. Prion diseases: pathogenesis and public health concerns. FEBS LETTERS, 529 (1): 17-21 OCT 2 2002.
    Abstract: Transmissible spongiform *encephalopathy* (TSE) agents or prions induce neurodegenerative fatal diseases in humans and in some mammalian species. Human TSEs include Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler- Scheinker syndrome, kuru and fatal familial insomnia. In animals, scrapie in *sheep* and *goats*, feline spongiform *encephalopathy*, transmissible mink *encephalopathy*, chronic wasting disease in wild ruminants, and bovine spongiform *encephalopathy* (BSE), which appeared in the UK in the mid-1980s [Wells, G.A.H. et al. (1987) Vet. Rec. 121, 419-420], belong to the TSE group. Prions have biological and physicochemical characteristics that differ significantly from those of other microorganisms; for example, they are resistant to inactivation processes that are effective against conventional viruses, including those that alter nucleic acid structure or function. Alternatively, infectivity is highly susceptible to procedures that modify protein conformation. Today, the exact nature of prions remains unknown even though it is likely that they consist of protein only. At the biochemical level, TSEs are characterised by the accumulation, within the central nervous system of the infected individual, of an abnormal isoform of a particular protein from the host, the prion protein [Prusiner, S.B. (1982) Science 216, 136-144]. TSEs are transmissible among their species of origin, but they can also cross the species barrier and induce chronic infection and/or disease in other species. Transmissibility has been proven in natural situations such as the outbreak of CJD among patients treated with pituitary-derived hormones and the appearance of BSE that affected UK cattle in the mid- 1980s. (C) 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies. DOCUMENT TYPE: Review

36. Dormont, D. (. *Prions*, *BSE* and food. INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, 78 (1-2): 181-189 Sp. Iss. SI SEP 15 2002.
    Abstract: Biochemical and biophysical properties of *prions* including possible inactivation methods are reviewed. Possible molecular markers of *transmissible* *spongiform* *encephalopathy* (TSE) and mechanisms behind infectivity and correlation with clinical symptoms are discussed. The risk of *Bovine* *Spongiform* *Encephalopathy* (*BSE*) for humans i.e. variant *Creutzfeldt*-Jakob Disease (cCJD) is addressed in detail. The consequences of the emergence of the new cCJD and the lack of information on the infectivity of cCJD at the clinical stage of the disease in relation to the need to reconsider the biological concepts currently used in

microbiology. (C) 2002 Elsevier Science B.V. All rights reserved. DOCUMENT TYPE: Article

376. Foster, J. D., Parnham, D., Chong, A., Goldmann, W., & Hunter N. (2001). Clinical signs, histopathology and genetics of experimental transmission of BSE and natural scrapie to sheep and goats. VETERINARY-RECORD, 148, 165-71.
Abstract: This paper compares the clinical signs, histopathology, detection of PrPSc protein and PrP genetics of the transmission of BSE to sheep and goats, with the effects of the transmission of natural scrapie from a brain homogenate from a single sheep. After intracerebral and oral inoculations, there were similarities in the clinical signs due to the two sources of infection, but there were differences in pathology at the end stage of disease and in the genotypes of the sheep which succumbed to the challenges. The incubation period of BSE was associated with the sheep PrP codon 171 gentoype, but the natural scrapie sources despite inducing disease only in known susceptible genotypes, showed no clear association with PrP genotype.

38. Fries, R., Eggers, T., Hildebrandt, G., Rauscher, K., Buda, S., & Budras, K. D. (2003). Autonomous nervous system with respect to dressing of cattle carcasses and its probable role in transfer of PrPres molecules. Journal of Food Protection, 66(5), 890-895.
Abstract: Pathogen prions are widely recognized as the causative agent in bovine spongiform encephalopathy (BSE) and other transmissible spongiform encephalopathies. However, more research on the possible transmission routes of this agent once it has reached the host is needed. There is evidence based on the anatomy and physiology of the autonomous nervous system (ANS), as well observations for different animal species, that the ANS might be involved in the axonal drainage of pathogen prions toward the central nervous system. IN this context, more attention should be paid to the cranial cervical ganglion, the stellate ganglion, the chain of paravertebral ganglia next to the first six thoracic vertebrae, the chain of the ganglia nexxt to the first six thoracic vertebrae, the chain of the paravertebral ganglia next to loin vertebrae 1 through 6, the vagus nerve in the neck region and in the mediastin, and the esopahgus (because of its close connection to the vagus nerve). For a more detailed risk analysis with respect to these tissues, the ANSs of animals having shown clinical signs of BSE might be examined to corroborate the evidence presented here. In the meantime, as a precautionary measure, the tissue addressed should be taken out of the human food chain, taken out of animal feed, and handled as if it were specificed risk material. It is technically possible to remove these parts during cutting dressing.

39. Gale, P. (2001). Developments in Microbiological Risk Assessment for Drinking Water. Journal of Applied Microbiology, 91(2), 191-205.

40. Gale, P., Young, C., & Lewin, K. (2000). Comment on 'Outbreak of Enteroviruses and Groundwater Contamination in Taiwan: Concept of Biomedical Hydrogeology' (Jean 1999) - a Question of Barriers and Epidemiology. Hydrogeology Journal, 8(3), 348-349.

41. Gangidi, R. R., Proctor, A., & Pohlman, F. W. (2003). Rapid determination of spinal cord content in ground beef by attenuated total reflectance fourier transform infrared spectroscopy. Journal of Food Science, 68(1), 124-7.

42. Garcia, F. L., Zahn, R., Riek, R., & Wuthrich, K. (2000). Nmr Structure of the Bovine Prion Protein. Proceedings of the National Academy of Sciences of the United States of America, 97(15), 8334-8339.
Abstract: The NMR structures of the recombinant 217-residue polypeptide chain C-terminal fragment, bPrP(121-230). include a globular domain extending from residue 125 to residue 227. a short flexible chain end of residues 228-230, and an N-terminal flexibly disordered 'tail' comprising 108 residues for the intact protein and 4 residues for bPrP(121-230), respectively. The globular domain contains three alpha-helices comprising the residues 144-154, 173-194 and 200-226, and a short antiparallel beta-sheet comprising the residues 128-131 and 161-164. The best-defined parts of the globular domain are the central portions of the helices 2 and 3. which are linked by the only disulfide bond in bPrP. Significantly increased disorder and mobility is observed for helix 1. the loop 166-172 leading from the beta-strand 2 to helix 2, the end of helix 2 and the following loop, and the

last turn of helix 3. Although there are characteristic local differences relative to the conformations of the murine and Syrian hamster prion proteins, the bPrP structure is essentially identical to that of the human prion protein. On the other hand, there are differences between bovine and human PrP in the surface distribution of electrostatic charges, which then appears to be the principal structural feature of the 'healthy' PrP form that might affect the stringency of the species barrier for transmission of prion diseases between humans and cattle.

43. Gavier-Widen, D., Wells, G. A. H., Simmons, M. M., Wilesmith, J. W. W., & Ryan, J. (2001). Histological Observations on the Brains of Symptomless 7-Year-Old Cattle. Journal of Comparative Pathology, 124(1), 52-59.
Abstract: The histological changes in the brains of 506 clinically normal 7-year-old cattle, which were part of a cohort study On maternal transmission of bovine spongiform encephalopathy, are described. Vacuolation of the white matter. of unknown aetiology, located particularly in the substantia nigra, was a frequent finding. Vacuolated neurons were commonly observed in the red nucleus (64.3% of the animals) and in the habenular nucleus (50.1%). Spheroids were round in 10.8% of the brains, most frequently in the vestibular nuclei. Cellular inflammatory infiltrates ill association with blood vessels occurred in 30% of the animals at various locations in the brain; their aetiology remains uncertain, but they may have reflected subclinical or latent infections. Mineralization of the wall of blood vessels, with proliferation of the intima, was observed frequently ill vessels of the internal capsule and was probably associated with ageing. The description of histological findings ill the brain of symptomless adult cattle in the present study provides a useful background for diagnostic bovine neuropathology. (C) 2001 Harcourt Publishers Ltd.

44. Ghani, A., Ferguson, N., Donnelly, C., & Anderson, R. M. (2003). Factors determining the pattern of the variant Creutzfeldt-Jakob Disease (vCJD) epidemic in Great Britain. The Royal Society.
Abstract: Following the emergence of a new variant of Creutzfeltd-Jakob (vCJD) disease six years ago, and the gradual rise in clinical cases, there has been increased speculation regarding the overall magnitude of this epidemic in Great Britain. In this paper, we explore the epidemiological factors and uncertainties determining the scale of this epidemic in light of the most recent data on reported vCJD mortality. OUr results demonstrate that, while the magnitude of this uncertainty has decreased dramatically over the past six years, it is still not possible to predict with any degree of accuracy the final magnitude of this epidemic, with the 95% confidence intercal for future cases being from 10 to 7000 deaths. However, short-term projections show that it is unlikely that a dramatic increase in case numbers will be observed in the next 2-5 years (95% confindence interval for 2 years: 10-80 cases, for 5 years: 10-200 cases). The results confirm significant age-dependent susceptibility/exposure to infection, with the likelihood profile demonstrating that those aged between 10a dn 20 years are at highest risk of infection. We also demonstrate how projections based on onset data may be substantially biased, and explore the sensitivity of results to assumptions concerning the exposure to bovine spongiform encephalopathy (BSE) and the incubation period distribution.

45. Giese, A., Bieschke, J., Eigen, M., & Kretzschmar, H. A. (2000). Putting Prions Into Focus: Application of Single Molecule Detection to the Diagnosis of Prion Diseases. Archives of Virology, 161-171.
Abstract: Prion diseases are characterized by the cerebral deposition of an aggregated pathological isoform of the prion protein (PrPSc) which constitutes the principal component of the transmissible agent termed prion. In order to develop a highly sensitive method for the detection of PrPSc aggregates in biological samples such as cerebrospinal fluid (CSF), we used a method based on Fluorescence Correlation Spectroscopy (FCS), a technique which allows detection of single fluorescently labeled molecules in solution. Within the FCS setup, fluorescent photons emitted by molecules passing: an open volume element defined by the beam of an excitation laser focussed into a diffraction-limited spot are imaged confocally onto a single photon counting detector. Aggregates of PrPSc could be labeled by coaggregation of probe molecules such as monomeric recombinant PrP or PrP-specific antibodies tagged with a fluorescent dye. In addition to slow diffusion,? labeled aggregates are characterized by high fluorescence intensity, which allows detection and quantification by analysis of fluorescence intensity distribution. To improve detection of rare target particles, the accessible volume element was increased by scanning for intensely fluorescent targets

(SIFT). To further improve sensitivity and specificity, two different probes were used simultaneously in a two-color setup. In a diagnostic model system of CSF spiked with purified prion rods, dual-color SIFT was more sensitive than Western blot analysis. In addition, a PrPSc-specific signal was also detected in a number of CSF samples derived from CJD patients but not in controls.

46. Glatzel M, Abela E, Maissen M, & Aguzzi A. (2003). Extraneural pathologic prion protein in sporadic Creutzfeldt-Jakob disease. NEW-ENGLAND-JOURNAL-OF-MEDICINE. NOV 6 2003; 349 (19) : 1812-1820.
    Abstract: BACKGROUND: In patients with sporadic Creutzfeldt-Jakob disease, pathologic disease-associated prion protein (PrP(sup Sc)) has been identified only in the central nervous system and olfactory-nerve tissue. Understanding the distribution of PrP(sup Sc) in Creutzfeldt-Jakob disease is important for classification and diagnosis and perhaps even for prevention. METHODS: We used a highly sensitive method of detection -- involving the concentration of PrP(sup Sc) by differential precipitation with sodium phosphotungstic acid, which increased the sensitivity of Western blot analysis by up to three orders of magnitude -- to search for PrP(sup Sc) in extraneural organs of 36 patients with sporadic Creutzfeldt-Jakob disease who died between 1996 and 2002. RESULTS: PrP(sup Sc) was present in the brain tissue of all patients. In addition, we found PrP(sup Sc) in 10 of 28 spleen specimens and in 8 of 32 skeletal-muscle samples. Three patients had PrP(sup Sc) in both spleen and muscle specimens. Patients with extraneural PrP(sup Sc) had a significantly longer duration of disease and were more likely to have uncommon molecular variants of sporadic Creutzfeldt-Jakob disease than were patients without extraneural PrP(sup Sc). CONCLUSIONS: Using sensitive techniques, we identified extraneural deposition of PrP(sup Sc) in spleen and muscle samples from approximately one third of patients who died with sporadic Creutzfeldt-Jakob disease. Extraneural PrP(sup Sc) appears to correlate with a long duration of disease.

47. Glatzel M, Ott PM, Linder T, Gebbers JO, Gmur A, Wust W, Huber G, Moch H, Podvinec M, Stamm B, & Aguzzi A. (2003). Human prion diseases: epidemiology and integrated risk assessment. LANCET-NEUROLOGY. DEC 2003; 2 (12) : 757-763.
    Abstract: Human prion disases are devastating and incurable, but are very rare. Fears that the bovine spongiform encephalopathy epizootic would lead to a large epidemic of its presumed human counterpart, variant Creutzfeldt-Jakob disease (vCJD), have not been realised. Yet a feeling of uncertainty prevails in the general public and in the biomedical world. The lack of data on the prevalence of asymptomatic carriers of vCJD compounds this uncertainty. In addition to this problem, Switzerland is currently faced with another issue of major public concern: a recent rise in the incidence of CJD. Here we examine the plausibility of several scenarios that may account for the increase in CJD incidence, including ascertainment bias due to improved reporting of CJD, iatrogenic transmission, and transmission of a prion zoonosis. In addition, we present the design and current status of a Swiss population-wide study of subclinical vCJD prevalence.

48. Goldwater, P. N. (2001). Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease: Implications for Australia. Medical Journal of Australia, 175(3), 154-158.
    Abstract: The bovine spongiform encephalopathy (BSE) epizootic developed in the United Kingdom in the mid-1980s. Feeding practices in the cattle industry amplified the causative prion, and meat contaminated with BSE entered the market. Human consumption of prion-contaminated meat led to the new zoonosis - variant Creutzfeldt-Jakob disease (vCJD). The UK BSE Inquiry published its report in October 2000; while praising policy decisions, it also documented failures in the execution of these policies, specifically delays and lack of rigour. Australia is in an excellent position to maintain its BSE- and scrapie-free status, but widespread active surveillance of neural and non-neural tissue from all species of farmed quadrupeds is needed.

49. Gonzalez, L., Martin, S., & Jeffrey, M. (2003). Distinct profiles of PrPd immunoreactivity in the brain of scrapie- and BSE-infected sheep: implications for differential cell targeting and PrP processing. Journal of General Virology, 84(1339-50), 11.
    Abstract: Previous studies have shown tha tthe patterns of disease-specific prion protein (PrPd) accumulation in the brain (the 'PrPd profile') of scrapie-affected sheep are mainly influenced by the source of scrapie agent. We have now extended those studies to investigate the effect of different

PrP antibodies on the PrPd profile of scrapie- and bovine spongiform encephalopathy (BSE)-affected sheep. Immunohistochemical examination of brains of 20 sheep was performed with four different PrP antibodies (P4, 521.7, 505.2 and R486), and the animals were allocated to four groups of five sheep each depending on the transmissible spongiform encephalopathy (TSE) agent source (two natural scrapie sources, SSBP/1 and BSE). Although the PrPd profiles depended on the antibody used, the four TSE sources could always be differentiated. Natural Suffolk scrapie showed the highest levels of glia-associated PrPd, natural Welsh Mountain scrapie uniquely had consistent vascular PrPd plaques, SSBP/1 produced the higest intracellular accumulations of PrPd and BSE led to moderate accumulation of all PrPd patterns except for vascular plaques. The variations in PrPd profile between TSE sources appeared to be the result of variations in cell tropism and in PrP processing. These processing differences are possibly associated with changes in PrPd conformation, and are manifest as differences in intracellular truncation and in release to the extracellular space of the abnormal protein. Moreover, variations in PrPd conformation would appear to be also influenced by the cell type supporting infection, arguing that it is modulated by the interaction between the infectious agent and the host.

50. Hagenaars, T. J., Ferguson, N. M., Donnelly, C. A., Ghani, A. C., & Anderson, R. M. (2000). Feed-Borne Transmission and Case Clustering of Bse. Proceedings of the Royal Society of London Series B-Biological Sciences, 267(1440), 205-215.

    Abstract: An unresolved issue in the epidemiology of bovine spongiform encephalopathy (BSE) in the UK is what precisely determines the degree to which cases of disease in cattle are clustered within herds throughout the course of the epidemic. This paper presents an analysis of feed-borne transmission at the herd level and tests various models of case-clustering mechanisms, associated with heterogeneity in exposure to infectious feed, against observed epidemic pattern. We use an age-structured metapopulation framework in which the recycling of animal tissue between herds via feed producers is explicitly described. We explore two alternative assumptions for the scaling with herd size of the within-herd risk of exposure of an animal to infectious material. We find that whereas exposure heterogeneity caused by variation in feed and offal processing methods and by variation in per-animal feed uptake can explain the pattern of case clustering seen in the BSE epidemic, exposure heterogeneity due to the aggregation of infectivity within feed cannot.

51. Hidaka S, & Liu SY. (2003). Effects of gelatins on calcium phosphate precipitation: a possible application for distinguishing bovine bone gelatin from porcine skin gelatin. Journal of Food Composition, 16(4), 477-483.

    Abstract: Our study on the effects :of different gelatins on calcium phosphate precipitation demonstrated that bovine bone gelatin and porcine skin gelatin could be-distinguished using.a pH drop method. The induction time was more remarkably increased by.the addition of bovine bone gelatin than in the case of porcine skin gelatin. The difference between :these two :gelatins was significant at :the concentrations of 0.5 and 2.0 mg/ml. The effects on hydroxyapatite transformation were biphasic. Gelatins increased the value at a lower concentration, but they decreased it after reaching a peak concentration. The peak concentration was 0.5 mg/ml for bovine bone gelatin, :but was 40 mg/ml for porcine skin gelatin. Two commercial gelatin products were studied. One had properties close to bovine gelatin, and :the other had properties close to porcine gelatin. This simple method may be useful in analyzing the origin of gelatin products. (C) 2003 Elsevier Science Ltd. All rights reserved.

52. Hill, A. F., & Collinge, J. (. Species-barrier-independent *prion* replication in apparently resistant species. APMIS, 110 (1): 44-53 JAN 2002.

    Abstract: *Prion* *diseases* of humans and animals are associated with the accumulation of an abnormal isoforin (PrPSc) of the host-encoded *prion* *protein* (PrPC). Transmission of these *diseases* between mammalian species is usually limited by a 'species barrier' which can be mediated by differences in primary sequence of the *prion* *protein* between donor and host species. Studies on species barriers usually rely on the development of clinical *disease* in inoculated animals as an assessment of susceptibility in a particular host. Recent studies by a number of groups have demonstrated that the absence of clinical symptoms does not necessarily exclude transmission of *prion* *disease* across a species barrier. Such results indicate that subclinical or carrier states exist in these *diseases*, which has public health implications regarding human exposure to *BSE* *prions* and iatrogenic transmission from apparently healthy humans. Here the issue of subclinical *Prion* *diseases* is reviewed and implication., are discussed. DOCUMENT TYPE: Article

53. Hill, A. F., Joiner, S., Linehan, J., Desbruslais, M., Lantos, P. L., & Collinge, J. (2000). Species-Barrier-Independent Prion Replication in Apparently Resistant Species. Proceedings of the National Academy of Sciences of the United States of America, 97(18), 10248-10253.
    Abstract: Transmission of prions between mammalian species is thought to be limited by a 'species barrier,' which depends on differences in the primary structure of prion proteins in the infecting inoculum and the host, Here we demonstrate that a strain of hamster prions thought to be nonpathogenic for conventional mice leads to prion replication to high levels in such mice but without causing clinical disease. Prions pathogenic in both mice and hamsters are produced. These results demonstrate the existence of subclinical forms of prion infection with important public health implications, both with respect to iatrogenic transmission from apparently healthy humans and dietary exposure to cattle and other species exposed to bovine spongiform encephalopathy prions, Current definitions of the species barrier, which have been based on clinical endpoints, need to be fundamentally reassessed.

54. Hilton, D. A. (2000). Vcjd - Predicting the Future? Neuropathology and Applied Neurobiology, 26(5), 405-407.
    Abstract: The recent emergence of variant Creutzfeldt-Jakob disease (vCJD) in the UK, and demonstration that vCJD is caused by the same prion strain that causes bovine spongiform encephalopathy, have led to concerns about the possibility of a human epidemic. Although only 79 cases of vCJD have occurred to date, it is likely that hundreds of thousands of infected cattle entered the human food chain in the late 1980s and early 1990s, and the average incubation period of vCJD is unknown. Mathematical models have not yet been able to give useful predictions of future numbers of cases, and in the absence of a blood test for vCJD, current attempts to reduce uncertainties about future numbers of cases are based on the accumulation of PrPSc in lymphoreticular tissues. Extensive lymphoreticular PrPSc accumulation has been seen in all cases of symptomatic vCJD so far examined, and in one case 8 months prior to the onset of symptoms. Animal models of prion disease suggest that lymphoreticular involvement occurs early in the incubation period and reliably predicts future neurological disease. Based on these data, large scale anonymous studies looking for PrP accumulation in surgically removed tonsillectomy and appendicectomy specimens are underway. Examination of the first 3000 specimens has not revealed any positive samples, but at the moment the significance of negative findings is uncertain. It is anticipated that by the time these studies are complete more data will be available on how early PrP can be demonstrated in lymphoreticular tissue in vCJD, which together with the results from examination of further samples, will allow some comment as to the likelihood of a large human vCJD epidemic.

55. Hoots, W. K., Abrams, C., & Tankersley, D. (2001). The Impact of Creutzfeldt-Jakob Disease and Variant Creutzfeldt-Jakob Disease on Plasma Safety. Transfusion Medicine Reviews, 15(2), 45-59.
    Abstract: Although the true risk of transmitting (classical) Creutzfeld-Jakob Disease (CJD) and variant CJD (vCJD) via transfusion is likely very minimal, a review of prions and the impact of these associated prion diseases is timely because of their current effect on safety policies in the blood-plasma industry. Various types of human and animal prion diseases are outlined and reviewed, with emphasis on the importance of cross-species transmission as is relevant for vCJD. Review of the prion theory focuses on the relationship of prions to disease pathophysiology, prion resistance to protein modification, and potential prion transmission. Causes (with emphasis on iatrogenic CJD) and diagnosis of clinical CJD are described and contrasted with the same for vCJD. The origin of vCJD, the pathophysiologic questions surrounding this condition, and the latest diagnostic tests and research are also reviewed. Comparison of transmission feasibility versus actual transmission of CJD or vCJD by blood or blood products is then explored. Reasons for the discrepancy between theoretic and actual transmission for CJD and the body of evidence provided by look-back studies are examined. When compared with CJD, reasons are provided for the higher theoretic risk of

transmitting vCJD by blood products. Studies evaluating transmission by blood products in animals are considered. Transfusion practices that include European and US criteria to prevent vCJD through brood products are reviewed. This includes the debate surrounding product leukoreduction, deferral of donors at high risk either for exposure to vCJD or for contracting CJD, targeted elimination of donor plasma, and how some policies may have contributed to product shortages. Copyright (C) 200f by W.B. Saunders Company.

56. Hunter, N., Foster, J., Chong, A., McCutcheon, S., Parnham, D., Eaton, S., MacKenzie, C., & Houston, F. (. *Transmission* of *prion* *diseases* by blood transfusion. JOURNAL OF GENERAL VIROLOGY, 83: 2897-2905 Part 11 NOV 2002.
Abstract: Attempts to detect infectivity in the blood of humans and animals affected with *transmissible* *spongiform* *encephalopathies* (TSEs or *prion* *diseases*) have often been inconclusive because of the limitations of cross-species bioassays and the small volumes of blood that can be injected by the intracerebral route. A model has been developed for the experimental study of TSE *transmission* by blood transfusion using sheep experimentally infected with *bovine* *spongiform* *encephalopathy* (*BSE*) or natural *scrapie* as donors and susceptible *scrapie*-free sheep as recipients. Donors and recipients of the same species greatly increase the sensitivity of the bioassay and in sheep large volumes of blood can be injected by the intravenous (i.v.) route. *Transmission* of *BSE* to a single animal using this approach was reported recently. This study confirms this result with a second *transmission* of *BSE* and four new cases of *transmission* of natural *scrapie*. Positive *transmissions* occurred with blood taken at pre-clinical and clinical stages of infection. Initial studies indicate that following such infection by the i.v. route, deposition of the abnormal *prion* *protein* isoform, PrPSc, in peripheral tissues may be much more limited than is seen following oral infection. These results confirm the risks of TSE infection via blood products and suggest that the measures taken to restrict the use of blood in the UK have been fully justified. DOCUMENT TYPE: Article

57. Irani, D. N., & Johnson, R. T. (2003). Diagnosis and prevention of bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. Annu Rev Med, 54, 305-19.
Abstract: An outbreak of bovine spongiform encephalopathy (BSE) arose in the United Kingdom as a result of prions entering and being recycled through the ruminant food chain. Humans have since developed a variant form of Creutzfeldt-Jakob disease (vCJD), also mostly in the United Kingdom, that occurs in younger individuals and causes prominent psychiatric and/or behavioral manifestations early in disease. Laboratory studies now provide strong evidence that the causative agent of BSE in cattle and vCJD in humans share a common origin. Because of a lack of information regarding the incubation period of vCJD and the number of people who may have been exposed, the future scope of this disease remains unknown. Control of the current and any future outbreaks in cattle requires strict measures to prevent contamination of the animal food chain with prions of any species. Prevention of human exposure mandates the avoidance of neural tissue in all human foods.

58. Ironside, J. W., McCardle, L., Horsburgh, A., Lim, Z., & Head, M. W. (. Pathological diagnosis of variant *Creutzfeldt*-Jakob *disease*. APMIS, 110 (1): 79-87 JAN 2002.
Abstract: The neuropathological and biochemical features of the 89 histologically confirmed cases of variant *Creutzfeldt*-Jakob *disease* (*vCJD*) diagnosed up to the end of October 2001 in the UK are reviewed. Histology of the central nervous system, lymphoid tissues and other organs was accompanied by immunocytochemistry and Western blot analysis of the *disease*-associated form of the *prion* *protein* (PrPRES). All patients with *vCJD* were methionine homozygotes at codon 129 of the PrP gene. The pathology of *vCJD* showed relatively uniform morphological and immunocytochemical characteristics, which were distinct from other forms of *CJD*. PrPRES accumulation was widespread in lymphoid tissues in *vCJD*, but was not identified in other non-neural tissues. PrPRES in *vCJD* brain tissue showed a uniform glycotype pattern distinct from sporadic *CJD*. Given the increasingly widespread occurrence of *bovine* *spongiform* *encephalopathy* in Europe and Asia, there is a major need for widespread *CJD* surveillance. This should be accompanied by a multidisciplinary laboratory approach to the investigation and diagnosis of all forms of *CJD*, with the need to investigate autopsy tissues from suspected cases by the histological and biochemical techniques described herein. DOCUMENT TYPE: Article

59. Jackman, R., & Schmerr, M. J. (2003). Analysis of the performance of antibody capture methods using fluorescent peptides with capillary zone electrophoresis with laser-induced fluorescence. Electrophoresis, 24(5), 892-6.

Abstract: A method to analyze the performance of an antibody capture method using fluorescent peptides by capillary zone electrophoresis using laser-induced fluorescence (CZE-LIF) for detection has been developed. Fluorescent peptides from the prion protein were synthesized and the corresponding antibodies were produced in rabbits against these peptides. The antibodies were used to capture the fluorescent peptides. The antibodies were then bound to protein A Sepharose. After elution, the amount of fluorescent peptide that was captured vs. the total amount placed in the assay was evaluated by CZE-LIF. Of the three peptides used in this evaluation, it was found that the recovery was approximately 25-35%. When the abnormal prion protein was prepared from scrapie-infected brain samples from hamsters and a sheep using the previously described extraction method and this method, the amount of abnormal prion protein that was measured in the fluorescence immunoassay correlated with amounts estimated from Western blot. We conclude that this method can be used to detect abnormal prion protein in a tissue sample.

60. Jackson, G. S., & Collinge, J. (2001). The molecular pathology of CJD: old and new variants. Mol Pathol, 54(6), 393-9.

Abstract: The study of prion disease has become an area of intense interest since experimental evidence emerged for the transmission of phenotypic variation without the involvement of a nucleic acid component. Additional impetus has come from the widespread concern that exposure to bovine spongiform encephalopathy contaminated material poses a distinct and, conceivably, a severe threat to public health in the UK and other countries. The occurrence of new variant Creutzfeldt-Jakob disease has dramatically highlighted the need for a precise understanding of the molecular basis of prion propagation. The molecular basis of prion strain diversity, previously a major challenge to the "protein only" model, can now be reconciled with propagation of infectious protein topologies. The conformational change known to be central to prion propagation, from a predominantly alpha-helical fold to one predominantly comprising beta-structure, can now be reproduced in vitro, and the ability of beta-PrP to form fibrillar aggregates provides a plausible molecular mechanism for prion propagation. Concomitantly, advances in the fundamental biology of prion disease have done much to reinforce the protein only hypothesis of prion replication.

61. Jacob, M., & Hellstrom, T. (2000). Policy Understanding of Science, Public Trust and the Bse-Cjd Crisis. Journal of Hazardous Materials, 78(1-3), 303-317.

Abstract: The article investigates how institutional factors can produce risk using the Bovine Spongiform Encephalopathy (BSE)-Creutzfeldt-Jakob Disease (CJD) crisis in Britain as a case example. The paper focuses on the way policymakers understand science, and the role of precaution in issues of high uncertainty. It is argued that the failure to fully appreciate the complexity of the BSE-CJD situation resided in institutional arrangements that predisposed decision makers to adopt a counter productive approach in handling situations of high scientific uncertainty on the policy level. The article will demonstrate how these factors played out in the BSE-CJD crisis. (C) 2000 Elsevier Science B.V. All rights reserved.

62. James, M., Crabbe, C., & Hepburne-Scott, H. W. (2001). Small heat shock proteins (sHSPs) as potential drug targets. Curr Pharm Biotechnol, 2(1), 77-111.

Abstract: Small heat shock proteins (sHSPs) belong to a family of 12- to 43-kDa proteins that are ubiquitous and are largely conserved in amino acid sequence among all organisms. The principal heat-shock proteins that have chaperone activity (that is, they protect newly made proteins from misfolding) belong to five conserved classes: HSP100, HSP90, HSP70, HSP60 and the small heat-shock proteins (sHSPs). The sHSPs (which include alpha crystallin) can form large multimeric structures and have a wide range of cellular functions, including endowing cells with thermotolerance in vivo and being able to act as molecular chaperones in vitro; sHSPs do this by forming stable complexes with folding -or unfolding--intermediates of their protein substrates, probably the molten globule. This paper includes: a brief survey of the chaperone family, the small heat shock protein superfamily, transcription of sHSPs, sequence comparisons and structural models of small heat shock proteins--structural elements as potential drug targets, sHSPs as chaperone-like proteins, alpha crystallin chaperone-like activity, conformational diseases--the role of alpha crystallin small heat shock protein superfamily proteins, post-translational modification and useful pharmacological agents. Functionality of small heat shock proteins--targets and diseases where pharmacologically active agents are of importance, alpha crystallin--small heat shock proteins and prion diseases: specific targets for diagnostic tests and drug development, details of some specific small heat shock proteins as drug targets, structural and functional implications for treatment.

63. Jeffrey, M., Martin, S., Gonzalez, L., Ryder, S. J., Bellworthy, S. J., & Jackman, R. (2001). Differential diagnosis of infections with the bovine spongiform *encephalopathy* (BSE) and scrapie agents in *sheep*. Journal of Comparative Pathology, 125(4), 271-84.
Abstract: Scrapie, bovine spongiform *encephalopathy* (BSE), and variant Creutzfeldt Jakob disease belong to the group of disorders called transmissible spongiform *encephalopathies* or prion diseases. The possibility that some *sheep* may be infected with the BSE agent is of human and animal health concern. Immunohistochemical methods were used to identify specific prion protein (PrP) peptide sequences in specific cell types of the brain and lymphoreticular system (LRS) of *sheep* with natural scrapie and Suffolk and Romney *sheep* infected experimentally with the BSE agent. Clinically affected and some pre-clinical cases of BSE infection could be distinguished from scrapie cases by the lesser amount of labelling of PrP containing the 84-102 amino-acid peptide sequences in phagocytic cells of the LRS and brain. Additionally, BSE- infected *sheep* had higher degrees of intra-neuronal PrP accumulation ill the brain, as detected by labelling for a range of PrP peptide sequences. These results suggest that there is strain-dependent processing of PrP in specific cell types within the nervous system and LRS which call be used to distinguish BSE- and scrapie-infected *sheep*.

64. Kamphues, J., Zentek, J., Oberthur, R. C., Flachowsky, G., & Coenen, M. (2001). Risk Assessment for Animal Derived Feedstuffs as Vectors for Bovine Spongiform Encephalopathy (Bse) in Germany. Part I: Comparative Risk Assessment for Animal Derived Feedstuffs. Deutsche Tierarztliche Wochenschrift, 108(7), 283-290.
Abstract: The occurrence of BSE cases in Germany after the ban of meat and bone meal for ruminant feed in 1994 requires a detailed investigation of animal derived feedstuffs regarding their specific risks as vectors for the disease. Accepting the theory that BSE is a prion transmitted disease, the theoretical infectious potential was calculated for animal derived feedstuffs. This calculation was based on the assumption, that risk material (brain, spinal cord) of one clinically diseased cattle was rendered in the process as established in Germany (133 degreesC, 3 bar, 20 min) or, alternatively, that one diseased animal was slaughtered resulting in normal processing of the by-products for human food production. From this risk assessment it became obvious that meat and bone meal was one, but probably not the most important source for the spreading of BSE. Taking into account the high sensitivity of calves it can be speculated that certain products, eg. from bone processing (bone meal) and fat melting (mixed animal fats), commonly used for the formulation of milk replacers, might have been more important as pathways. As it can't be excluded retrospectively that infected meat and bone meal was imported from the UK, this non-calculable influence may have relativated the significance of the other products. The calculation model underlines that efficient removal of specified risk material (brain, spinal cord) and adequate processing (133 degreesC, 3 bar, 20 min) or alternatively other equivalent treatments of fats are prerequisites for minimising the risk of feed borne transmission of BSE by animal derived feedstuffs. The epidemiological consequences are part of a subsequent paper.

65. King, C. Y. (2001). Supporting the Structural Basis of Prion Strains: Induction and Identification of [Psi] Variants. Journal of Molecular Biology, 307(5), 1247-1260.
Abstract: The [PSI] genetic element, which enhances the nonsense suppression efficiency in the yeast Saccharomyces cerevisiae, is thought to be amyloid-like aggregates of the Sup35 protein, and to self-propagate by a prion-like mechanism. Analogous to strains of the mammalian prion, variants of [PSI], with different nonsense suppression efficiencies and mitotic stabilities, can be isolated from the same yeast genetic background. Ln the framework of the 'protein-only' hypothesis, variants of prion are assumed to be distinct conformers of the same prion polypeptide. This study aims to provide further support for the structural basis of [PSI] variation. Three variants of [PSI] were

induced and distinguished by a panel of 11 single point mutations of the Sup35 protein. The variant phenotypes are intrinsically associated with [PSI] elements, presumably structurally different amyloids, rather than produced from variations in the genetic background. Differential incorporation to [PSI] variants of a Sup35 point mutation as well as N and C-terminally truncated Sup35 fragments is further demonstrated in vivo, suggesting that distinct patches of amino acid residues are involved in the assembly of [PSI] variants. These results establish a method for [PSI] variant-typing and indicate that heritable variations of amyloid structures can be derived from the same polypeptide. (C) 2001 Academic Press.

66. Kingombe, C. I. B., Luthi, E., Schlosser, H., Howald, D., Kuhn, M., & Jemmi, T. (2001). A Pcr-Based Test for Species-Specific Determination of Heat Treatment Conditions of Animal Meals as an Effective Prophylactic Method for Bovine Spongiform Encephalopathy. Meat Science, 57(1), 35-41.
Abstract: Efficiency of animal waste sterilization prescribed by the European Union and Switzerland was verified using a pork-based ELISA and two PCR assays (tRNA(Glu)/cytochrome b specific for vertebrates; bovine species-specific cytochrome b mitochondrial genome). A total of 204 samples of feedingstuffs were analysed including reference materials subjected to known heat treatments. Both ELISA and PCR assays were able to detect poorly heat-treated feedingstuffs if there was enough pork-based material present. The proposed species-specific PCR test, however, showed a higher sensitivity and specificity as it specifically detected bovine material. Nevertheless, the PCR assay will not detect bovine material in properly heat-treated feeds as the DNA is too fragmented. It is, however, very useful as a rapid, sensitive, and specific method for the routine screening of animal meals with regard to prophylaxis of BSE. (C) 2000 Elsevier Science Ltd. All rights reserved.

67. Klass, M. R., Hodges, S., Sayers, R., & Clarke, J. (2003). A test for transmissible spongiform encephalopathy. American Biotechnology Laboratory .
Abstract: Because of the various routes of infectivity and the presumed passage of BSE to humans, all TSEs are being scrutinized more closely. For example, while it is not believed that scrapie passes from sheep to humans, it is not known if CWD in deer and elk can pass to humans. This concern has caused a great deal of interest in the testing of harvested animals meant for human consumption as a means of safeguarding against possible human infection. One way to test for TSEs is via the detection of the infectious agent, PrPSc, by immunological methods. Because of the large number of samples involved and the relative rapidity of the test, an enzyme linked immunosorbant assay (ELISA) format is clearly beneficial. The Enfer TSE test (manufactured by Enfer Scientific, Newbridge, Ireland; distributed by Abbott Laboratories, Abbot Park , IL) is an ELISA that has the advantages of simplicity, rapidity, high throughput, sensitivity, safety, and availability in kit form. The test has been approved by the European Commission and is currently marketed in Europe for the detection of BSE in cattle. It is also currently used in Ireland to screen sheep for scrapie and is being evaluated in the U.S. as an aid in the diagnosis of CWD in deer and elk. This paper describes use of the assay for the detection of PrPSc in the central nervous system of cattle, sheep, and deer.

68. Korth, C., Kaneko, K., Groth, D., Heye, N., Telling, G., Mastrianni, J., Parchi, P., Gambetti, P., Will, R., Ironside, J., Heinrich, C., Tremblay, P., DeArmond, S. J., & Prusiner, S. B. (2003). Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. Proc Natl Acad Sci U S A, 100(8), 4784-9.
Abstract: Transgenic (Tg) mouse lines that express chimeric mouse-human prion protein (PrP), designated MHu2M, are susceptible to prions from patients with sporadic Creutzfeldt-Jakob disease (sCJD). With the aim of decreasing the incubation time to fewer than 200 days, we constructed transgenes in which one or more of the nine human residues in MHu2M were changed to mouse. The construct with murine residues at positions 165 and 167 was expressed in Tg(MHu2M,M165V,E167Q) mice and resulted in shortening the incubation time to approximately 110 days for prions from sCJD patients. The construct with a murine residue at position 96 resulted in lengthening the incubation time to more than 280 days for sCJD prions. When murine residues 96, 165, and 167 were expressed, the abbreviated incubation times for sCJD prions were abolished. Variant CJD prions showed prolonged incubation times between 300 and 700 days in Tg(MHu2M) mice on first passage and incubation times of approximately 350 days in Tg(MHu2M,M165V,E167Q) mice. On second and third passages of variant CJD prions in

Tg(MHu2M) mice, multiple strains of prions were detected based on incubation times and the sizes of the protease-resistant, deglycosylated PrP(Sc) fragments. Our discovery of a previously undescribed chimeric transgene with abbreviated incubation times for sCJD prions should facilitate studies on the prion species barrier and human prion diversity.

69. Kretzschmar HA. (2003). Diagnosis of prion diseases. CLINICS-IN-LABORATORY-MEDICINE. MAR 2003; 23 (1) : 109-128,VIII.

    Abstract: This article reviews the various techniques and methodologies for the recognition and diagnosis of clinical and neuropathologic variants of human prion diseases.

70. Lange B, Alter T, Froeb A, & Lucker E. (2003). Molecular biological detection of tissues of central nervous system in meat products. BERLINER-UND-MUNCHENER-TIERARZTLICHE-WOCHENSCHRIFT. NOV-DEC 2003; 116 (11-12) : 467-473.

    Abstract: In principle, molecular biological methods can be used for the detection of specified risk material (SRM) in meat products. We were able to identify suitable target mRNAs for the marker proteins glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) of tissues of the central nervous system (CNS). The selected primers for GFAP ('GFAP(87)') and MBP ('MBP51') facilitated the detection of CNS in non-heated and heated standards of emulsion type (and raw) sausages with defined addition of brain by reverse transcription and polymerase chain reaction (RT-PCR). Stability of the mRNA proved to be given over a minimum of 48 hours. First results indicate an even higher stability of the target mRNAs over time and an ample stability against meat technological influences such as storage, temperature, and ripening. RT-PCR with GFAP(87) facilitates the detection of CNS of all relevant slaughter animals in meat products. Thus it can be applied in food labeling control. Using RT-PCR and MBP51 as primer we were able to detect selectively the CNS of bovines, ovines and caprines but not CNS of porcines and poultry. Thus we have a second RT-PCR detection procedure for CNS in meat products which, however, yields results equivalent to the legal definition of SRM. The further development of these molecular biological methods would be of considerable importance for routine food control and reduction of human TSE-exposure risk.

71. Langeveld, J. P., Wang, J. J., Van de Wiel, D. F., Shih, G. C., Garssen, G. J., Bossers, A., & Shih, J. C. (2003). Enzymatic degradation of prion protein in brain stem from infected cattle and sheep. J Infect Dis, 188(11), 1782-9.

    Abstract: Prions-infectious agents involved in transmissible spongiform encephalopathies-normally survive proteolytic and mild protein-destructive processes. Using bacterial keratinase produced by Bacillus licheniformis strain PWD-1, we tested conditions to accomplish the full degradation of prion protein (PrP) in brain-stem tissue from animals with bovine spongiform encephalopathy and scrapie. The detection of PrPSc, the disease-associated isoform of PrP, in homogenates was done by Western blotting and various antibodies. The results indicated that only in the presence of detergents did heat pretreatment at >100 degrees C allow the extensive enzymatic breakdown of PrPSc to a state where it is immunochemically undetectable. Proteinase K and 2 other subtilisin proteases, but not trypsin and pepsin, were also effective. This enzymatic process could lead to the development of a method for the decontamination of medical and laboratory equipment. The ultimate effectiveness of this method of prion inactivation has to be tested in mouse bioassays.

72. Lasmezas, C. I. (2003). Putative functions of PrP(C). Br Med Bull, 66, 61-70.

    Abstract: While the exact function of the cellular prion protein (PrP(C)) remains unknown, there are several leads due to increasing knowledge on the localisation and interaction of PrP(C) with other molecules. This chapter will concentrate on these aspects. Identified ligands of PrP(C) mainly belong to the categories of heat-shock proteins, membrane-bound receptors, or heparan sulphates. The possible synaptic role of PrP(C) has been exemplified by electrophysiological findings in PrP(o/o) mice and the studies of PrP(C) as a copper-binding molecule that could regulate the copper content of the synaptic cleft. The latter property of PrP(C) may also endow PrP(C) with the activity of a copper-dependent superoxide dismutase. Binding of PrP(C) to signalling molecules suggests a role as a transmitter of information from the extracellular milieu to the cell and a trigger for a molecular cascade. This agrees with new data on PrP(C) receptors and the role of PrP(C) in cell

survival.

73. LeBlanc, A. C., & Roucou, X. (2003). Toxicity and protection in prions. Science, 301(5630), 168-9; author reply 168-9.

74. Legname, G., Nelken, P., Guan, Z. Y., Kanyo, Z. F., DeArmond, S. J., & Prusiner, S. B. (. *Prion* and doppel *proteins* bind to granule cells of the cerebellum. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 99 (25): 16285-16290 DEC 10 2002.
Abstract: We reported that expression of the cellular *prion* *protein* (PrPC) rescues doppel (Dpl)-induced cerebellar degeneration in mice. To search for *protein*(s) that mediate this process, we fused the C-termini of mouse (Mo) PrP and Dpl to the Fc portion of an IgG. Although both MoPrP-Fc and MoDpl-Fc bound to many regions of the brain, we observed restricted binding to granule cells in the cerebellum, suggesting a scenario in which granule cells express a *protein* that mediates Dpl- induced neurodegeneration. Because granule cells do not express PrPC, it seems unlikely that MoPrP-Fc binding reflects a ligand that is involved in the conversion of PrPC into PrPSc, the *disease*-causing isoform. In contrast, the dominant-negative MoPrP(Q218K)-Fc not only binds to granule cells but also binds to neurons of the molecular layer where PrPC is expressed. These findings raise the possibility that the cells of the molecular layer express an auxiliary *protein*, provisionally designated *protein* X, which is involved in *prion* formation and is likely to be distinct from the *protein* that mediates Dpl-incluced degeneration. Although the binding of the dominant-negative MoPrP(Q218)-Fc to cells in the molecular layer expressing PrPC is consistent with a scenario for the binding of MoPrP(Q218K)-Fc to *protein* X, the absence of PrPSc deposition in the molecular layer requires that PrPSc, once formed there, be readily transported to the cerebellar white matter where PrPSc is found. Identifying the ligands to which PrP-Fc, Dpl-Fc, and dominant- negative PrP bind may provide new insights into the functions of PrPC and Dpl as well as the mechanism of PrPSc formation. DOCUMENT TYPE: Article

75. Liberski, P. P., & Jaskolski, M. (. *Prion* *diseases*: a dual view of the *prion* hypothesis as seen from a distance. ACTA NEUROBIOLOGIAE EXPERIMENTALIS, 62 (3): 197-226 2002.
Abstract: We review the historical background and principles of the *prion* theory in its current shape. We showed that most of data may be still interpreted dually according to the *protein* only hypothesis and according to the theory in which additional component is necessary to comprise the infectivity. The enormous impact of structural biological studies is also stressed. DOCUMENT TYPE: Review

76. Lucker, E., Hardt, M., & Groschup, M. H. (. Detection of CNS and PrPSc in meat products. BERLINER UND MUNCHENER TIERARZTLICHE WOCHENSCHRIFT, 115 (3-4): 111-117 MAR-APR 2002.
Abstract: Several methods for the detection of tissues of the central nervous system (CNS) in meat products have been developed and partly validated for use in official food control as pertaining to human *BSE*-exposure risk. So far, however, methods for the detection of abnormal *prion* *protein* (PrPs,) were not evaluated for their potential applicability to the matrix of heat treated meat products. We developed a micro technological procedure for the preparation of meat products suitable for high security laboratories as masses were 6 to 8 orders of magnitude lower than in conventional meat technology. Thus it was possible to produce standard micro sausages containing defined amounts of *bovine* *BSE*-positive brain. This material showed all characteristics of normal meat products and a homogeneous distribution of brain as indicated by NSE and GFAP western immunoblotting and GFAP immunometric analyses. Using a commercially available and certified immunometric assay for detection of PrPSe in untreated brain it was possible to detect *BSE*-positive CNS down to a content of 0.25% in heat treated meat products. We found a high correlation between PrPSc OD-values and CNS content and linearity up to 10% CNS. In 30 samples of retail meat products no sample transgressed the official cut off value for untreated *bovine* brain. Further studies are needed to show whether an increase of sensitivity in PrPSc detection from the meat product matrix is possible, in particular by optimisation of the extraction procedure. DOCUMENT TYPE: Article

77. Mabbott NA, & Bruce ME. (2003). Prion disease: bridging the spleen-nerve gap. NATURE-MEDICINE. DEC 2003; 9 (12) : 1463-1464.

Abstract: Infection of tissues outside the nervous system occurs in a number of prion diseases, but sporadic Creutzfeldt- Jakob disease in humans has not been considered one of them. A clinical study of Swiss patients with sporadic disease reconsiders this assumption, and a second study examines the spread of infection between follicular dendritic cells and nerves.

78. Mabbott NA, Young J, McConnell I, & Bruce ME. (2003). Follicular dendritic cell dedifferentiation by treatment with an inhibitor of the lymphotoxin pathway dramatically reduces scrapie susceptibility. JOURNAL-OF-VIROLOGY. JUN 2003; 77 (12) : 6845-6854.

Abstract: Transmissible spongiform encephalopathies (TSEs) may be acquired peripherally, in which case infectivity usually accumulates in lymphoid tissues before dissemination to the nervous system. Studies of mouse scrapie models have shown that mature follicular dendritic cells (FDCs), expressing the host prion protein (PrPc), are critical for replication of infection in lymphoid tissues and subsequent neuroinvasion. Since FDCs require lymphotoxin signals from B lymphocytes to maintain their differentiated state, blockade of this stimulation with a lymphotoxin beta receptor-immunoglobulin fusion protein (LTbetaR-Ig) leads to their temporary dedifferentiation. Here, a single treatment with LTbetaR-Ig before intraperitoneal scrapie inoculation blocked the early accumulation of infectivity and disease-specific PrP (PrPSc) within the spleen and substantially reduced disease susceptibility. These effects coincided with an absence of FDCs in the spleen for ca. 28 days after treatment. Although the period of FDC dedifferentiation was extended to at least 49 days by consecutive LTbetaR-Ig treatments, this had little added protective benefit after injection with a moderate dose of scrapie. We also demonstrate that mature FDCs are critical for the transmission of scrapie from the gastrointestinal tract. Treatment with LTbetaR-Ig before oral scrapie inoculation blocked PrPSc accumulation in Peyer's patches and mesenteric lymph nodes and prevented neuroinvasion. However, treatment 14 days after oral inoculation did not affect survival time or susceptibility, suggesting that infectivity may have already spread to the peripheral nervous system. Although manipulation of FDCs may offer a potential approach for early intervention in peripherally acquired TSEs, these data suggest that the duration of the treatment window may vary widely depending on the route of exposure.

79. Macknight, C. (2001). Clinical Implications of Bovine Spongiform Encephalopathy. Clinical Infectious Diseases, 32(12), 1726-1731.

Abstract: Bovine spongiform encephalopathy (BSE) is a new prion disease that was first identified in the United Kingdom in 1987. Its appearance was likely caused by changes in the rendering process used to produce a meat and bone supplement for cattle, changes that allowed this prion to enter the bovine food supply. Despite measures that were made to reduce the risk to humans, a new variant of Creutzfeldt-Jakob disease appeared in the mid-1990s and has been linked to BSE. Although the extent of the disease's impact on humans is not yet known, current estimates predict that there will be 136,000 cases of this fatal disease by the year 2040. The risk to humans of medications produced with bovine materials, gelatin, and blood transfusion is unknown.

80. Manuelidis L. (2003). Transmissible encephalopathies: Speculations and realities. VIRAL-IMMUNOLOGY. 2003; 16 (2) : 123-139.

Abstract: Virtually all transmissible encephalopathies (TSEs), such as scrapie, CJD, and BSE, are caused by a type of infectious particle that remains enigmatic. The language of prion theory supersedes the reality of what is, and what is not known. This review questions the predictive value, consistency and accuracy of this now dominant assumption. Many people believe the normal cellular prion protein (PrP) self-converts into an infectious amyloid protein or prion. Although the amyloidogenic capacity of proteins is well established, the concept of an infectious protein without nucleic acid was 'revolutionary.' Diverse experiments have repeatedly shown, however, that this protein alone, in any form, is incapable of reproducing transmissible infection. In contrast, the infectious agent copurifies with many other molecules, including nucleic acids, while it separates from the majority of PrP. The infectious particle has a homogeneous viral size of similar to25 nm, and infectivity is markedly reduced by conditions that disrupt viral core components but do not disrupt multimers of PrP amyloid. Additionally, the infectious agent replicates to high levels before any PrP abnormalities can be detected. Hence, we initially proposed that PrP changes are part of the host's pathologic response to high levels of infectious agent, but not the agent itself. Newer data clarifying a role for myeloid cells in the spread of infection, the unique character of two different agent strains propagated in a single animal, and the demonstration of long nucleic acids in a variety of simplified high titer preparations continue to raise serious questions for the prion hypothesis. Moreover, the epidemic spread of TSEs, and the activation of host innate immune mechanisms by infection, further indicate these agents are recognizably foreign, and probably viral.

81. Masters, C. L. (2001). The Emerging European Epidemic of Variant Creutzfeldt-Jakob Disease and Bovine Spongiform Encephalopathy: Lessons for Australia. Medical Journal of Australia, 174(4), 160-161.

82. Mathews, D., & Unwin, D. H. (2001). Quantitative Cerebral Blood Flow Imaging in a Patient With the Heidenhain Variant of Creutzfeldt-Jakob Disease. Clinical Nuclear Medicine, 26(9), 770-773.
Abstract: Purpose: This report describes quantitative SPECT cerebral blood flow (CBF) changes in a patient with the confirmed Heidenhain variant of Creutzfeldt-Jakob disease. Materials and Methods: A 60-year-old man reported visual disturbances, including left hemianopsia. An electroencephalogram, magnetic resonance imaging, cerebral arteriogram, and quantitative SPECT CBF imaging with Xe-133, with and without acetazolamide, were performed. After the patient's death, an autopsy was performed. Results: The electroencephalographic findings were nonspecific, those of magnetic resonance imaging were normal, and the cerebral arteriogram showed minimal atherosclerosis. However, the SPECT scan revealed marked depression of perfusion in the parietal and occipital cortices that did not change with the administration of acetazolamide. Autopsy results were consistent with CJD. Conclusions: Although there is no consistent pattern of CBF changes that is characteristic of Creutzfeldt-Jakob disease, the Heidenhain variant of that disease may be an exception. Clinical symptoms include profound changes in vision, and the pattern of CBF changes in the patient described here included depression of perfusion in areas of primary and associated visual cortex. Also of interest is the finding that although other imaging did not reveal an abnormality, the CBF changes were marked. In addition, vasoreactivity is poor in response to acetazolamide, a finding that occurs only late in other neuronal degenerative conditions such as Alzheimer's disease.

83. May, B. C., Fafarman, A. T., Hong, S. B., Rogers, M., Deady, L. W., Prusiner, S. B., & Cohen, F. E. (2003). Potent inhibition of scrapie prion replication in cultured cells by bis-acridines. Proc Natl Acad Sci U S A, 100(6), 3416-21.
Abstract: Prion diseases are characterized by an accumulation of PrP(Sc), a misfolded isoform of the normal cellular prion protein, PrP(C). We previously reported the bioactivity of acridine-based compounds against PrP(Sc) replication in scrapie-infected neuroblastoma cells and now report the improved potency of bis-acridine compounds. Bis-acridines are characterized by a dimeric motif, comprising two acridine heterocycles tethered by a linker. A library of bis-(6-chloro-2-methoxy-acridin-9-yl) and bis-(7-chloro-2-methoxy-benzo[b][1,5]naphthyridin-10-yl) analogs was synthesized to explore the effect of structurally diverse linkers on PrP(Sc) replication in scrapie-infected neuroblastoma cells. Structure-activity analysis revealed that linker length and structure are important determinants for inhibition of prion replication in cultured scrapied cells. Three bis-acridine analogs, (6-chloro-2-methoxy-acridin-9-yl)-(3-[4-[3-(6-chloro-2-methoxy-acridin-9-y lamino)-propyl]-piperazin-1-yl]-propyl)-amine, N,N'-bis-(6-chloro-2-methoxy-acridin-9-yl)-1,8-diamino-3,6-dioxaoctane, and (1-[[4-(6-chloro-2-methoxy-acridin-9-ylamino)-butyl]-[3-(6-chloro-2-methox y-acridin-9-ylamino)-propyl]-carbamoyl]-ethyl)-carbamic acid tert-butyl ester, showed half-maximal inhibition of PrP(Sc) formation at 40, 25, and 30 nM, respectively, and were not cytotoxic to uninfected neuroblastoma cells at concentrations of 500 nM. Our data suggest that bis-acridine analogs may provide a potent alternative to the acridine-based compound quinacrine, which is currently under clinical evaluation for the treatment of prion disease.

84. Mead, S., Beck, J., Dickinson, A., Fisher, E. M. C., & Collinge, J. (2000). Examination of the Human Prion Protein-Like Gene Doppel for Genetic Susceptibility to Sporadic and Variant Creutzfeldt-Jakob Disease. Neuroscience Letters, 290(2), 117-120.
Abstract: A novel human gene named Doppel (DPL) that has homology to the prion protein gene (PRNP) has recently been identified on chromosome 20p. By automated sequencing we have found

a common (M174T, 48%) and an uncommon coding polymorphism. The polymorphic frequency of the M174T allele was examined in cases of variant and sporadic Creutzfeldt-Jakob Disease and compared with the frequency in the normal UK population. In sharp distinction to the M129V polymorphism of PRNP we have not found any evidence of disease association nor is there any association with age of onset, disease duration, or prion protein (PrPSc) strain type. (C) 2000 Elsevier Science Ireland Ltd. All rights reserved.

85. Mishra, R. S., Bose, S., Gu, Y., Li, R., & Singh, N. (2003). Aggresome formation by mutant prion proteins: the unfolding role of proteasomes in familial prion disorders. J Alzheimers Dis, 5(1), 15-23.
Abstract: Although familial prion disorders are a direct consequence of mutations in the prion protein gene, the underlying mechanisms leading to neurodegeneration remain unclear. Potential pathogenic mechanisms include abnormal cellular metabolism of the mutant prion protein (PrP(M)), or destabilization of PrP(M) structure inducing a change in its conformation to the pathogenic PrP-scrapie (PrP(Sc)) form. To further clarify these mechanisms, we investigated the biogenesis of mutant PrP V203I and E211Q associated with Creutzfeldt-Jakob disease, and PrP Q212P associated with Gerstmann-Straussler-Scheinker syndrome in neuroblastoma cells. We report that all three PrP(M) forms accumulate similarly in the cytosol in response to proteasomal inhibition, and finally assemble as classical aggresomes. Since the three PrP(M) forms tested in this report are distinct, we propose that sequestration of misfolded PrP(M) into aggresomes is likely a general response of the cellular quality control that is not specific to a particular mutation in PrP. Moreover, since PrP has the remarkable ability to refold into PrP(Sc) that can subsequently replicate, PrP(M) sequestered in aggresomes may cause neurotoxicity by both direct and indirect pathways; directly through PrP(Sc) aggregates, and indirectly by depleting normal PrP, through induction of a cellular stress response, or by other undefined pathways. On the other hand, sequestered PrP(M) may be relatively inert, and cellular toxicity may be mediated by early intermediates in aggresome formation. Taken together, these observations demonstrate the role of proteasomes in the pathogenesis of familial prion disorders, and argue for further explanation of its mechanistic details.

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Abstract: A familial prion disorder with a proline to leucine substitution at residue 102 of the prion protein (PrP(102L)) is typically associated with protease-resistant PrP fragments (PrP(Sc)) in the brain parenchyma that are infectious to recipient animals. When modeled in transgenic mice, a fatal neurodegenerative disease develops, but, unlike the human counterpart, PrP(Sc) is lacking and transmission to recipient animals is questionable. Alternate mice expressing a single copy of PrP(102L) (mouse PrP(101L)) do not develop spontaneous disease, but show dramatic susceptibility to PrP(Sc) isolates from different species. To understand these discrepant results, we studied the biogenesis of human PrP(102L) in a cell model. Here, we report that cells expressing PrP(102L) show decreased expression of the normal 18-kDa fragment on the plasma membrane. Instead, a 20-kDa fragment, probably derived from transmembrane PrP ((Ctm)PrP), accumulates on the cell surface. Because the 20-kDa fragment includes an amyloidogenic region of PrP that is disrupted in the 18-kDa form, increased surface expression of 20-kDa fragment may enhance the susceptibility of these cells to PrP(Sc) infection by providing an optimal substrate, or by amplifying the neurotoxic signal of PrP(Sc). Thus, altered susceptibility of PrP(101L) mice to exogenous PrP(Sc) may be mediated by the 20-kDa (Ctm)PrP fragment, rather than PrP(102L) per se.

87. Murphy, C., Breen, C., Rogers, M., & Giese, M. (2001). Interferon Gamma and Prostaglandin in Bse-Infected Cattle. Cytokine, 13(3), 169-173.
Abstract: Suspect field cases of BSE infection (displaying clinical signs) were examined for possible alterations of cytokine/autacoid plasma levels and were compared to control cases (not displaying clinical signs of BSE infection). Interferon gamma (IFN-gamma) plasma levels were demonstrated as being elevated in all suspected field cases of BSE infection (irrespective of BSE status; determined via postmortem histopathological examination). We demonstrated that plasma IFN-gamma levels were significantly (P<0.005) higher in suspect cases of BSE infection than in control cases. BSE-positive prostaglandin-E-2, (PGE(2)) plasma levels were demonstrated as being elevated

1.25-fold above BSE-negative cases and 2.22-fold above control cases. No significant (P>0.5) increase in PGE(2) plasma levels was recorded between BSE-positive and -negative. IFN-gamma and PGE(2) plasma levels were examined using commercially available ELISA assay. The results presented in this publication are the first demonstration of alteration in immune state in animals with BSE. (C) 2001 Academic Press.

88. Narang, H. K. (2001). Lingering Doubts About Spongiform Encephalopathy and Creutzfeldt-Jakob Disease. Experimental Biology and Medicine, 226(7), 640-652.
Abstract: Bovine spongiform encephalopathy (BSE) is an infectious disease and has been transmitted orally to many other animals, including humans. There is clear evidence of maternal transmission, although disagreement on the source of the BSE agent remains. The current theories link the origin of BSE to common scrapie in sheep. Twenty different strains of the scrapie agent have been Isolated from sheep. A search of the literature indicates two distinct clinical syndromes In sheep, both of which have been called scrapie. I have designated these Type I (the common type), which exhibits itchiness and lose their wool, and Type II, which exhibits trembling and ataxia. Sheep inoculated with BSE develop Type II scrapie and they exhibit trembling. When cattle or mink are injected with the Type I strain, only a few will develop a clinical disease. By contrast, no clinical disease has so far been shown in cattle or mink by feeding them with Type I-infected sheep brains. However, either by injecting or feeding with the BSE strain, 100% of calves and mink develop the clinical disease. Evidence suggests that Type II is the cause of BSE, Identical clinical signs of Type II trembling are found in kuru and many of the recent cases of Creutzfeldt-Jakob disease. The BSE agent has caused spongiform encephalopathies (SEs) in domestic cats, tigers, and in some species of ruminants in toes. The nature of the BSE agent remains unchanged when passaged through a range of species, Irrespective of their genetic make up, demonstrating that variations in the host PrP gene are not a major factor in the susceptibility to the BSE agent. Since more than 85 zoo animals of many species have been diagnosed with SEs, from these studies it seems reasonable to conclude that the BSE agent can infect almost all mammalian species, Including humans. For eradication of BSE and to reduce the risk of infection to humans, the development of a vaccine against BSE Is suggested. Such a possibility should be fully explored.

89. Nitsch, P., & Wachsamnn, G. (2001). Detection of Nerval Tissues (Brain & Spinal Cord) in Comminuted Meat by Using the Cholesterol-Testkit 'Enzym. Bioanalysis Cholesterin/R-Biopharm'. Fleischwirtschaft, 81(3), 76-78.
Abstract: The cholesterol-testkit 'Enzym.BioAnalysis Cholesterin/R-Biopharm' is used as screening test for the detection of nerval tissues in meat products. Nerval tissues (esp. brain) should significantly increase the cholesterol content of meat products. The test is commonly used in a double analysis. Following aspects were investigated: Sensitivity, specifity, variation of results and stabilty of the test to detect nerval tissues in meat products. It was shown, that the test did not detect pigbrain at concentrations of 0.5% and 1% in comminuted raw meat or meat products. Spinal cord of pig was detected from 0.5% on. At low concentrations (spinal cord <1% resp. brain <5%) the values are variing a lot, so that there is an overlap between contaminated and pure samples. Therefore at least 10 runs of the test at one sample must be done and the data must be variafied by statistical routines (ANOVA etc.). A distinction between the addition of spinal cord or brain is not possible.

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Abstract: Conformational conversion of proteins in disease is likely to be accompanied by molecular surface exposure of previously sequestered amino-acid side chains. We found that induction of beta-sheet structures in recombinant prion proteins is associated with increased solvent accessibility of tyrosine. Antibodies directed against the prion protein repeat motif, tyrosine-tyrosine-arginine, recognize the pathological isoform of the prion protein but not the normal cellular isoform, as assessed by immunoprecipitation, plate capture immunoassay and flow cytometry. Antibody binding to the pathological epitope is saturable and specific, and can be created in vitro by partial

denaturation of normal brain prion protein. Conformation-selective exposure of Tyr-Tyr-Arg provides a probe for the distribution and structure of pathologically misfolded prion protein, and may lead to new diagnostics and therapeutics for prion diseases.

91. Pastoret, P. P., Gouffaux, M., Saegerman, C., Roels, S., Dechamps, P., Thiry, E., & Vanopdenbosch, E. (2001). Rapid Immunological Diagnosis of Transmissible Spongiform Encephalopathies. Annales De Medecine Veterinaire, 145(3), 164-+.
Abstract: The development of rapid tests for the post mortem diagnosis of bovine spongiform encephalopathy made possible to extend the detection to bovine carcasses older than 30 months in slaughterhouses. This article reviews the rapid immunological tests and the measures enforced in Belgium to improve the control.

92. Peretz, D., Williamson, R. A., Legname, G., Matsunaga, Y., Vergara, J., Burton, D. R., DeArmond, S. J., Prusiner, S. B., & Scott, M. R. (. A change in the conformation of *prions* accompanies the emergence of a new *prion* strain. NEURON, 34 (6): 921-932 JUN 13 2002.
Abstract: To investigate the role of the pathogenic *prion* *protein* (PrPSc) in controlling susceptibility to foreign *prions*, two Syrian hamster (SHa) *prion* strains, Sc237 and DY, were transmitted to transgenic mice expressing chimeric SHa/mouse PrP genes, Tg(MH2M). First passage of SHa(Sc237) *prions* exhibited prolonged incubation times, diagnostic of a species barrier. PrPSc of the new MH2M(Sc237) strain possessed different structural properties from those of SHa(Sc237), as demonstrated by relative conformational stability measurements. This change was accompanied by a *disease* phenotype different from the SHa(Sc237) strain. Conversely, *transmission* of SHa(DY) *prions* to Tg(MH2M) mice showed no species barrier, and the MH2M(DY) strain retained the conformational and *disease*-specific properties of SHa(DY). These results suggest a causal relationship between species barriers, changes in PrPSc conformation, and the emergence of new *prion* strains. DOCUMENT TYPE: Article

93. Polak, M. P., & Zmudzinski, J. F. (2001). Bse as Zoonosis. Medycyna Weterynaryjna, 57(1), 5-8.
Abstract: This paper reviews current research on links between the appearance of BSE in cattle and variants of Creutzfeldt-Jakob disease in humans. The paper presents scientific evidence which shows that agents causing vCJD and CJD are different while vCJD and BSE agents are identical. The possible risk for humans caused by animal food consumption is presented.

94. Priola, S. A., Chabry, J., & Chan, K. M. (2001). Efficient Conversion of Normal Prion Protein (Prp) by Abnormal Hamster Prp Is Determined by Homology at Amino Acid Residue 155. Journal of Virology, 75(10), 4673-4680.
Abstract: In the transmissible spongiform encephalopathies, disease is closely associated with the conversion of the normal proteinase K-sensitive host prion protein (PrP-sen) to the abnormal proteinase K-resistant form (PrP-res). Amino acid sequence homology between PrP-res and PrP-sen is important in the formation of new PrP-res and thus in the efficient: transmission of infectivity across species barriers. It was previously shown that the generation of mouse PrP-res was strongly influenced by homology between PrP-sen and PrP-res at amino acid residue 138, a residue located in a region of loop structure common to PrP molecules from many different species. In order to determine if homology at residue 138 also affected the formation of PrP-res in a different animal species, we assayed the ability of hamster PrP-res to convert a panel of recombinant PrP-sen molecules to protease-resistant PrP in a cell-free conversion system. Homology at amino acid residue 138 was not critical for the formation of protease resistant hamster PrP. Rather, homology between PrP-sen and hamster PrP-res at amino acid residue 155 determined the efficiency of formation of a protease-resistant product induced by hamster PrP-res. Structurally, residue 155 resides in a turn at the end of the first alpha helix in hamster PrP-sen; this feature is not present in mouse PrP-sen. Thus, our data suggest that PrP-res molecules isolated from scrapie-infected brains of different animal species have different PrP-sen structural requirements for the efficient formation of protease-resistant PrP.

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96. Priola SA, Caughey B, & Chesebro B. (2003). Toxicity and protection in prions - Response. SCIENCE-. JUL 11 2003; 301 (5630) : 169.

97. Qin KF, Coomaraswamy J, Mastrangelo P, Yang Y, Lugowski S, Petromilli C, Prusiner SB, Fraser PE, Goldberg JM, Chakrabartty A, & Westaway D. (2003). The PrP-like protein doppel binds copper. JOURNAL-OF-BIOLOGICAL-CHEMISTRY, 278(11), 8888-8896.
Abstract: Doppel (Dpl) is a glycosylphosphatidylinositol-anchored protein expressed in the testis. It exhibits 26% sequence identity with the prion protein (PrP) but lacks the octarepeat region implicated as the major copperbinding domain. Contrary to expectations, Cu(II) induced a 26% reduction in the intrinsic fluorescence of Dpl(27-154) and a calculated K-d for a single-site model of 0.16 +/- 0.08 muM. Other metals had minimal effects on fluorescence quenching. Matrix-assisted laser desorption ionization mass spectrometry of a Dpl peptide revealed binding of copper (but not other metals) to the helical alphaB/B'- loop-alphaC subregion of Dpl. Fluorescence quenching and equilibrium dialysis analyses of this Dpl(101-145) peptide were compatible with a binding site of K-d = 0.4 muM. Diethylpyrocarbonate footprinting (Qin, K., Yang, Y., Mastrangelo, P., and Westaway, D. (2002) J. BioL Chem. 277, 1981-1990) of Dpl(27-154) defined one residue/molecule was protected by copper from die! thylpyrocarbonate adduct formation, and reiteration of this analysis with Dpl(101-145) suggested that His(131) may contribute to Cu(II) binding. Taken together, our data indicate that the alpha-helical region of mouse Dpl possesses a selective copper-binding site with a submicromolar Kd and perhaps one or more lower affinity sites. Although metallated forms of Dpl might exist in vivo, analyses of Tg(Dpl)10329 mice were inconsistent with reports that Dpl expression is associated with increased carbonylation and nitrosylation of brain proteins. Thus, rather than comprising an important source of free radical damage, copper binding may serve to modulate the activity, stability, or localization of the Dpl protein.

98. Race, R. E., Raines, A., Baron, T. G. M., Miller, M. W., Jenny, A., & Williams, E. S. (. Comparison of abnormal *prion* *protein* glycoform patterns from *transmissible* *spongiform* *encephalopathy* agent-infected deer, elk, sheep, and cattle. JOURNAL OF VIROLOGY, 76 (23): 12365-12368 DEC 2002.
Abstract: Analysis of abnormal *prion* *protein* glycoform patterns from *chronic* *wasting* *disease* (CWD)-affected deer and elk, *scrapie*-affected sheep and cattle, and cattle with *bovine* *spongiform* *encephalopathy* failed to identify patterns capable of reliably distinguishing these *transmissible* *spongiform* *encephalopathy* *diseases*. However, PrP- res patterns sometimes differed among individual animals, suggesting infection by different or multiple CWD strains in some species. DOCUMENT TYPE: Article

99. Raymond, G. J., Bossers, A., Raymond, L. D., O'rourke, K. I., Mcholland, L. E., Bryant, P. K., Miller, M. W., Williams, E. S., Smits, M., & Caughey, B. (2000). Evidence of a Molecular Barrier Limiting Susceptibility of Humans, Cattle and Sheep to Chronic Wasting Disease. Embo Journal, 19(17), 4425-4430.
Abstract: Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of deer and elk, and little is known about its transmissibility to other species. An important factor controlling interspecies TSE susceptibility is prion protein (PrP) homology between the source and recipient species/genotypes. Furthermore, the efficiency with which the protease-resistant PrP (PrP-res) of one species induces the in vitro conversion of the normal PrP (PrP-sen) of another species to the protease-resistant state correlates with the cross-species transmissibility of TSE agents. Here we show that the CWD-associated PrP-res (PrPCWD) of cervids readily induces the conversion of recombinant cervid PrP-sen molecules to the protease-resistant state in accordance with the known transmissibility of CWD between cervids, In contrast, PrPCWD-induced conversions of human and bovine PrP-sen were much less efficient, and conversion of ovine PrP-sen was intermediate. These results demonstrate a barrier at the molecular level that should limit the susceptibility of these non-cervid species to CWD.

100. Ricketts MN, & Brown P. (2003). Transmissible spongiform encephalopathy update and implications for blood safety. CLINICS-IN-LABORATORY-MEDICINE. MAR 2003; 23 (1) : 129-137,VIII,IX.
Abstract: This article reviews the evidence regarding the risk of transmission of the human

transmissible spongiform encephalopathies (TSEs) through transfusion and the implications for blood safety. At this time, the accumulated evidence does not support the implementation of measures targeted against the risk of transfusion transmission of sporadic, familial, or iatrogenic Creutzfeldt-Jakob disease (CJD). Evolving information about variant CJD (vCJD), however, suggests that policy makers need to consider implementing measures to protect against exposure to vCJD, if such measures themselves do not lead to decreased blood safety. Surveillance of TSEs and investigation of the risk of transfusion transmission must continue in order to provide further refinements in blood safety policy.

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Abstract: This article discussed the implications of the structures of PrP and Dpl--with their unusual folds containing N-terminal flexible tails and C-terminal globular domains--to the physiologic functions of PrPC and Dpl, and investigations of a possible structural basis of familial human TSEs. Further relations between TSEs and the PrP structure would include the species barrier of TSEs (which seems to be associated with species-specific structural characteristics of PrPC [25,39,67]), and the conformational transition from PrPC to PrPSc using, for example, molecular dynamic simulations [68,69]. Due to the lack of knowledge on physiologic functions of PrPC, however, and the remaining uncertainty about the exact role of the PrP in TSE pathology, it appears that most or all of the physiologically relevant structure-function correlations of PrPC have yet to be identified.

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Abstract: Richard Race reviews the recently published book 'How the Cows turned mad' by Maxime Schwartz, a molecular biologist at the Pasteur Institute in Paris. The book develops the history of TSEs as a mystery novel, poses questions and describes scientific advances and basic science.

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Abstract: Feline spongiform encephalopathy (FSE), a transmissible spongiform encephalopathy or prion disease of cats, first reported in Great Britain in 1990, is believed to result from the consumption of food contaminated by the agent of bovine spongiform encephalopathy (BSE). The accumulation of PrP in non-neural tissues of cats diagnosed as suffering from FSE was investigated by immunohistochemistry. In the majority of the cats no disease-specific PrP was detected in lymphoid tissues. Small amounts of PrP were detected in the spleen of only two of 13 samples examined, in Peyer's patches of one of the two cases for which suitable material was available, but in the myenteric plexus of all four cats in which sections of intestine were examined. In addition PrP immunostaining was found in the kidney of all the cats with FSE whose kidneys were examined.

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Abstract: Prions are the infectious agents responsible for transmissible spongiform encephalopathies. The principal component of prions is the glycoprotein PrPSc, which is a conformationally modified isoform of a normal cell-surface protein called PrPC (ref. 1). During the time between infection and the appearance of the clinical symptoms, minute amounts of PrPSc replicate by conversion of host PrPC, generating large amounts of PrPSc aggregates in the brains of diseased individuals. We aimed to reproduce this event in vitro. Here we report a procedure involving cyclic amplification of protein misfolding that allows a rapid conversion of large excess PrPC into a protease-resistant, PrPSc-like form in the presence of minute quantities of PrPSc template. In this procedure, conceptually analogous to polymerase chain reaction cycling, aggregates formed when PrPSc is incubated with PrPC are disrupted by sonication to generate multiple smaller units for the continued formation of new PrPSc. After cyclic amplification more than 97% of the protease-resistant PrP present in the

sample corresponds to newly converted protein. The method could be applied to diagnose the presence of currently undetectable prion infectious agent in tissues and biological fluids, and may provide a unique opportunity to determine whether PrPSc replication results in the generation of infectivity in vitro.

106. Safar, J. G., Scott, M., Monaghan, J., Deering, C., Didorenko, S., Vergara, J., Ball, H., Legname, G., Leclerc, E., Solforosi, L., Serban, H., Groth, D., Burton, D. R., Prusiner, S. B., & Williamson, R. A. (. Measuring *prions* causing *bovine* *spongiform* *encephalopathy* or *chronic* *wasting* *disease* by immunoassays and transgenic mice. NATURE BIOTECHNOLOGY, 20 (11): 1147-1150 NOV 2002.
Abstract: There is increasing concern over the extent to which *bovine* *spongiform* *encephalopathy* (*BSE*) *prions* have been transmitted to humans, as a result of the rising number of variant *Creutzfeldt*-Jakob *disease* (*vCJD*) cases. Toward preventing new *transmissions*, diagnostic tests for *prions* in livestock have been developed using the conformation-dependent immunoassay (CDI), which simultaneously measures specific antibody binding to denatured and native forms of the *prion* *protein* (PrP). We employed high-affinity recombinant antibody fragments (recFab) reacting with residues 95-105 of *bovine* (Bo) PrP for detection and another recFab that recognizes residues 132-156 for capture in the CDI. We report that the CDI is capable of measuring the *disease*-causing PrP isoform (PrPSc) in *bovine* brainstems with a sensitivity similar to that of end-point titrations in transgenic (Tg) mice expressing BoPrP. *Prion* titers were similar to10(7) ID50 units per gram of *bovine* brainstem when measured in Tg(BoPrP) mice, a figure similar to10 times greater than that determined by bioassay in cattle and similar to10,000 greater than in wild-type mice. We also report substantial differences in BoPrP(Sc) levels in different areas of the obex region, where neuropathology has been consistently observed in cattle with *BSE*. The CDI was able to discriminate between PrPSc from *BSE*-infected cattle and Tg(BoPrP) mice as well as from *chronic* *wasting* *disease* (CWD)- infected deer and elk. Our findings argue that applying the CDI to livestock should considerably reduce human exposure to animal *prions*. DOCUMENT TYPE: Article

107. Saiz, A., & Pereira, A. (2001). Prions and Blood Donation. Medicina Clinica, 116(11), 422-424.

108. Schwagele, F. (2001). Information About Bse - Possibilities and Limits of Analysis. Fleischwirtschaft, 81(4), 143-145.
Abstract: Reliable procedures for the detection of components of cattle and tissue of the central nervous system (CNS) in meat products have gained significance with respect to BSE. Analytical methods for species identification are based either on protein or DNA, e.g. an immunological test like ELISA, polymerase chain reaction (PCR) in combination with analysis of restriction fragment length polymorphism (RFLP), hybridisation by means of DNA-probes (Southern-Blotting) and iso-electric-focusing (IEF) techniques. Concerning the different animal species in meat products the detection limits of the mentioned techniques are varying between several percent and traces. With the exception of IEF these methods record beef as well as other components originating from cattle in corresponding products. A test offered as a service by a private laboratory uses cholesterol, neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP) for the identification of CNS in meat products. Applying this method an animal species specific differentiation of CNS is not possible and the question is still open, if the method provides a tool to differentiate between CNS and peripheral nervous tissue in muscle.

109. Scott, M. R., Supattapone, S., Nguyen, H. O. B., Dearmond, S. J., & Prusiner, S. B. (2000). Transgenic Models of Prion Disease. Archives of Virology, 113-124.
Abstract: There is growing concern that bovine spongiform encephalopathy (BSE) may have passed from cattle to humans, resulting in similar to 70 cases of an atypical, variant CJD (vCJD) in teenagers and young adults. We report here that transgenic (Tg) mice expressing full-length bovine (Bo) PrP serially propagate BSE prions and that there is no species barrier for transmission from cattle to Tg(BoPrP) mice. Surprisingly, these same mice were also highly susceptible to vCJD and natural sheep scrapie. The incubation times (similar to 250 d), neuropathology, and PrPS' isoforms in TE(BoPrP) mice inoculated with vCJD and BSE brain extracts were indistinguishable and differed

dramatically from those seen in these mice injected with natural scrapie. In efforts to identify PrP sequences required for prion formation, we found that a redacted prion protein of only 106 amino acids (PrP106) containing two large deletions supported prion propagation. In Tg(PrP106) mice, an artificial transmission barrier for the passage of full-length mouse prions was diminished by the coexpression of full-length wt MoPrPC, suggesting that wt MoPrP acts in trans to accelerate the replication of 'miniprions' containing PrP(Sc)106. Following a single passage (similar to 300 d) in Tg(PrP106) mice, the miniprions efficiently transmitted disease to Tg(PrP106) mice after only similar to 66 days. Our findings with Tg(BoPrP) mice provide compelling evidence that prions from cattle with BSE have infected humans and caused fatal neurodegeneration, the unique features of miniprions offer new insights into the mechanism of prion replication, and the trans-acting effects of full-length PrP coexpression suggest a new approach to the development of even more efficient animal models for prion diseases.

110. Solassol J, Crozet C, & Lehmann S. (2003). Prion propagation in cultured cells. BRITISH-MEDICAL-BULLETIN. 2003; 66 : 87-97.
Abstract: Cell cultures represent relevant and useful experimental models of transmissible spongiform encephalopathies (TSEs). They are able to promote, upon subpassaging, stable and persistent replication of PrPSc as well as infectivity. To date, only a few cell culture models permissive to prion replication are available. Among them, mouse neuroblastoma cell lines (N2a) are most commonly used. While they correspond to homologous models supporting propagation of mouse-adapted scrapie strains, recent studies have reported heterologous models sensitive to natural occurring disease. Infected cell culture models have provided some valuable insights into the biogenesis of PrPSc in terms of conversion, subcellular localisations, physiopathological consequences and species-barrier determinants. They have also contributed to the screening and the study of possible therapeutic compounds and to the development of new strategies for the investigation of TSE-specific diagnostic markers.

111. Stack, M. J., Chaplin, M. J., & Clark, J. (2002). Differentiation of prion protein glycoforms from naturally occurring *sheep* scrapie, *sheep*-passaged scrapie strains (CH1641 and SSBP1), bovine spongiform *encephalopathy* (BSE) cases and Romney and Cheviot breed *sheep* experimentally inoculated with BSE using two monoclonal antibodies. ACTA NEUROPATHOLOGICA, 104(3), 279.
Abstract: A panel of ruminant brain tissues were subjected to a Western immunoblotting technique using two monoclonal antibodies (mAbs). The resultant prion protein (PrP) glycoforms showed that three distinctions can be made between natural ovine scrapie cases and *sheep* experimentally inoculated with bovine spongiform *encephalopathy* (BSE). Differentiation between BSE-infected cattle and natural cases of *sheep* scrapie was also possible using these two antibodies. There were subtle differences in the molecular weight positions of the diglycosylated, mono- glycosylated and unglycosylated forms of the abnormal PrP (PrPsc) associated with these ruminant transmissible spongiform *encephalopathies*. In particular, a distinct difference for the unglycosylated protein band was observed. For ovine scrapie samples, this band was noticeably of a higher molecular weight than that found for brain samples from the Romney and Cheviot breed *sheep* infected with BSE and, to a lesser degree, higher than that observed for bovine BSE samples. Using the comparison of glycoform ratios, the technique provided a distinction between the *sheep* experimentally infected with BSE and natural cases of *sheep* scrapie but did not provide a distinction between natural cases of bovine BSE and ovine scrapie. The *sheep*- passaged CH1641 scrapie strain gave molecular weights similar to, but not identical to BSE, and a glycoform ratio similar to ovine scrapie cases. The SSBP1 experimental scrapie strain gave molecular weights that were akin to natural scrapie cases but the glycoform ratio was different to that found for all the other samples. When mAb P4 was substituted for mAb 6H4 in the technique, only the natural scrapie samples and SSBP1 gave strong signals. BSE in *sheep* and the CH1641 strain gave weak reactions and PrPsc from BSE-infected cattle could not be detected at all. The results suggest that this combination of molecular weight and glycoform ratio analyses, and differentiation with two specific antibodies could be used to provide a possible screening test for BSE in the UK *sheep* flock, if confirmed as accurate by bioassay and lesion profile analysis in mice inoculated with brain tissue from suspect field cases. DOCUMENT TYPE: Article

112. Stoiber, J., Ruckhofer, J., Lametschwandtner, A., Muss, W., Hitzl, W., Weikinger, K., & Grabner, G. (2001). Eurosol Versus Fetal Bovine Serum-Containing Corneal Storage Medium. Cornea, 20(2), 205-209. Abstract: Purpose. To evaluate the usability of Eurosol, a new medium-term corneal storage medium without components of bovine origin. Methods. Ten pairs of human donor corneas were placed in tissue culture at 31 degreesC for 7, 14, 21, 28, or 35 days. One cornea of each pair was cultivated in conventional storage medium on Earls' minimum essential medium base containing 2% fetal bovine serum; the other one was stored in Eurosol. Corneas were examined with inverse light microscopy; corneal thickness was measured; and scanning electron microscopy was performed. Results. No significant difference in corneal thickness and endothelial cell count was found at any time. Scanning electron microscopy showed a complete endothelial cell layer on all corneas. Conclusion. The findings indicate a potential clinical applicability of the tested serum-free medium-term storage medium, offering a safer alternative to conventional media containing fetal bovine serum.

113. Taylor, D. (2002). Inactivaion of the BSE agent. Vol. 325, 75-76. Abstract: In the studies carried out so far, the BSE agent has proved to be just as resistant as other TSE agents to inactivation by procedures such as autoclaving or exposure to sodium hydroxide that are effective with conventional microorganisms. However, in comon with other TSE agents, the BSE agent appears to be effectively inactivated by exposure to sodium hypochlorite solutions containing high levels of available chlorine. Not surprisingly, the BSE agent has been found to survive a least some of the rendering processes that were used to process tissues discarded by abattoirs in the EU during the early l980s. Despoite the survival of BSE infectivity after autoclaving or exposure to sodium hydroxide, it is known that combining these procedures results in a very reliable degree of inactivation for TSE agents generally. The combination of heat and alkali has also been found that the exposure of acid-treated bone (which is free from any obvious remains of fatty or proteinaceous tissue) to 0.3 M sodium hydroxide for two hours knocks out any residual BSE infectivity. The cite this article: D. Taylor, C.R. Biologies 325 (2002) 75-76. © 2002 Académie des Sciences/Ediitions scientifiques et médicals Elsevier SAS

114. Taylor, D. M. (2003). Inactivation of TSE agents: safety of blood and blood-derived products. TRANSFUSION-CLINIQUE-ET-BIOLOGIQUE. FEB 2003; 10 (1) : 23-25. Abstract: Evidence relating to whether the blood of individuals with sporadic Creutzfeldt-Jakob disease is infectious is discussed. The conclusion, is that this is unproven. Similar consideration is given to the blood of individuals with variant Creutzfeldt-Jakob disease; it is concluded that there is no convincing evidence that the blood is infectious but reasons for caution are presented. There is discussion regarding factors that add to the safety of plasma-derived therapeutic products, including the capacity of the manufacturing processes to inactivate or remove infectivity by the chemical and physical processes involved. There is extended discussion regarding the inactivation of these types of agents and the few reliable options available in worst-case scenarios such as the processing of instruments used neurosurgically on known or suspected cases. The most effective method is exposure to 1 M sodium hydroxide during autoclaving at 121 degreesC. The inappropriateness of applying any of the most effective methods to blood and blood-products because they are harsh and denaturing is discussed. Nevertheless, such procedures have potential application to the plant used in the manufacture of plasma-products. Evidence is presented which suggests that even more modest treatments (the use of lower concentrations of sodium hydroxide at lower temperatures) are effective when applied to surfaces that are-free from any tissue contamination, as is the case with plant used to manufacture plasma-derived products. This evidence has come from studies carried out by the gelatin manufacturers of Europe regarding the capability of their manufacturing systems to inactivate the causal agent of bovine spongiform encephalopathy. (C) 2003 Editions scientifiques et medicales Elsevier SAS. All rights reserved.

115. Taylor, D. M., Fernie, K., Steele, P. J., & Somerville, R. A. (2001). Relative Efficiency of Transmitting Bovine Spongiform Encephalopathy to Riii Mice by the Oral Route. Veterinary Record, 148(11), 345-346.

116. Taylor DM, & Woodgate SL. (2003). Rendering practices and inactivation of transmissible spongiform encephalopathy agents. REVUE-SCIENTIFIQUE-ET-TECHNIQUE-DE-L-OFFICE-

INTERNATIONAL-DES-EPIZOOTIES. APR 2003; 22 (1) : 297-310.
Abstract: The authors describe the historic form of rendering and provide details on present-day practice. Possible future directions for the rendering industry are considered. The role of rendered meat-and-bone meal (MBM) as a dietary supplement in propagating the United Kingdom (UK) epidemic of bovine spongiform encephalopathy (IBSE) is discussed, together with the role of MBM in spreading BSE outside the UK. Evidence that customarily used rendering processes did not substantially inactivate the agents of IBSE or scrapie is presented. In addition, the influence that the abandonment of solvent extraction (as an adjunct to rendering) in the UK might have had on BSE infectivity levels in MBM is discussed. The BSE-related safety of tallow and by-products of tallow are considered. Data that associate the IBSE agent with a new variant form of Creutzfeldt-Jakob disease in humans, predominantly but not exclusively, in the UK, are also discussed.

117. Thackray, A. M., Madec, J. Y., Wong, E., Morgan-Warren, R., Brown, D. R., BaronT, & Bujdoso, R. (2003). Detection of bovine spongiform encephalopathy, ovine scrapie prion-related protein (PrPsc) and normal PrPc by monoclonal antibodies raised to cooper-refolded prion protein. Biochemical Journal, 370, 81-90.

118. Turner, M. L. (2001). Variant Creutzfeldt-Jakob Disease and Blood Transfusion. Current Opinion in Hematology, 8(6), 372-379.
Abstract: Variant Creutzfeldt-Jakob disease (vCJD) was first described in the United Kingdom in 1996 and is thought to have been transmitted from cattle infected with bovine spongiform encephalopathy probably via the food chain. Thus far just over 100 definite or probable clinical cases have been described, though the number of people currently infected and the eventual size and geographic distribution of any future clinical epidemic remain uncertain. There is little evidence that sporadic CJD is transmitted by blood transfusion. However, the same cannot necessarily be assumed to apply to the new variant strain of disease in which involvement of peripheral lymphoid tissues has been demonstrated. In the face of uncertainty surrounding the risk of transmission of vCJD by blood products, blood transfusion services in a number of countries have implemented precautionary policies, though whether in the long term these will prove to have been necessary or sufficient remains to be seen. (C) 2001 Lippincott Williams & Wilkins, Inc.

119. Uptain, S. M., & Lindquist, S. (. *Prions* as *protein*-based genetic elements. ANNUAL REVIEW OF MICROBIOLOGY, 56: 703-741 2002.
Abstract: Fungal *prions* are fascinating *protein*-based genetic elements. They alter cellular phenotypes through self-perpetuating changes in *protein* conformation and are cytoplasmically partitioned from mother cell to daughter. The four *prions* of Saccharomyces cerevisiae and Podospora anserina affect diverse biological processes: translational termination, nitrogen regulation, inducibility of other *prions*, and heterokaryon incompatibility. They share many attributes, including unusual genetic behaviors, that establish criteria to identify new *prions*. Indeed, other fungal traits that baffled microbiologists meet some of these criteria and might be caused by *prions*. Recent research has provided notable insight about how *prions* are induced and propagated and their many biological roles. The ability to become a *prion* appears to be evolutionarily conserved in two cases. [PSI+] provides a mechanism for genetic variation and phenotypic diversity in response to changing environments. All available evidence suggests that *prions* epigenetically modulate a wide variety of fundamental biological processes, and many await discovery. DOCUMENT TYPE: Review

120. Venters, G. A. (2001). New Variant Creutzfeldt-Jakob Disease: the Epidemic That Never Was. British Medical Journal, 323(7317), 858-861.

121. Wadsworth JDF, Joiner, S., Hill, A. F., Campbell, T. A., Desbruslais, M., Luthert, P. J., & Collinge, J. (2001). Tissue Distribution of Protease Resistant Prion Protein in Variant Creutzfeldt-Jakob Disease Using a Highly Sensitive Immunoblotting Assay. Lancet, 358(9277), 171-180.
Abstract: Background Variant Creutzfeldt-Jakob disease (vCJD) has a pathogenesis distinct from other forms of human prion disease: disease-related prion protein (PrPSc) is readily detectable in lymphoreticular tissues. Quantitation of risk of secondary transmission, and targeting of risk

reduction strategies, is limited by lack of knowledge about relative prion titres in these and other peripheral tissues, the unknown prevalence of preclinical vCJD, and a transmission barrier which limits the sensitivity of bioassay. We aimed to improve immunoblotting methods for high sensitivity detection of PrPSc to investigate the distribution of PrPSc in a range of vCJD tissues. Methods We obtained tissues at necropsy from four patients with neuropathologically confirmed vCJD and from individuals without neurological disease. Tissues were analysed by sodium phosphotungstic acid precipitation of PrPSc and western blotting using high sensitivity enhanced chemiluminescence. Findings We could reliably detect PrPSc in the equivalent of 50 nL 10% vCJD brain homogenate, with a maximum limit of detection equivalent to 5 nl. PrPSc could be detected in tissue homogenates when present at concentrations 10(4)-10(5) fold lower than those reported in brain. Tonsil, spleen, and lymph node were uniformly positive for PrPSc at concentrations in the range of 0.1-15% of those found in brain: the highest concentrations were consistently seen in tonsil. PrPSc was readily detected in the retina and proximal optic nerve of vCJD eye at levels of 2.5 and 25%, respectively of those found in brain. Other peripheral tissues studied were negative for PrPSc with the exception of low concentrations in rectum, adrenal gland, and thymus from a single patient with vCJD. vCJD appendix and blood (Buffy coat fraction) were negative for PrPSc at this level of assay sensitivity. Interpretation We have developed a highly sensitive immunoblot method for detection of PrPSc in vCJD tissues that can be used to provide an upper limit on PrPSc concentrations in peripheral tissues, including blood, to inform risk assessment models. Rectal and other gastrointestinal tissues should be further investigated to assess risk of iatrogenic transmission via biopsy instruments. Ophthalmic surgical instruments used in procedures involving optic nerve and the posterior segment populations; of the eye, in particular the retina, might represent a potential risk for iatrogenic transmission of vCJD. Tonsil is the tissue of choice for diagnostic biopsy and for population screening of surgical tissues to assess prevalence of preclinical vCJD infection within the UK and other populations.

122. Weissmann, C., Enari, M., Klohn, P. C., Rossi, D., & Flechsig, E. (2002). Transmission of prions. Proc Natl Acad Sci U S A, 99 Suppl 4, 16378-83.
Abstract: The "protein only" hypothesis states that the infectious agent causing transmissible spongiform encephalopathies is a conformational isomer of PrP, a host protein predominantly expressed in brain, and is strongly supported by many lines of evidence. Prion diseases are so far unique among conformational diseases in that they are transmissible, not only experimentally but also by natural routes, mainly by ingestion. A striking feature of prions is their extraordinary resistance to conventional sterilization procedures, and their capacity to bind to surfaces of metal and plastic without losing infectivity. This property, first observed in a clinical setting, is now being investigated in experimental settings, both in animals and in cell culture.

123. Weissmann, C., & Flechsig, E. (2003). PrP knock-out and PrP transgenic mice in prion research. Br Med Bull, 66, 43-60.
Abstract: Spongiform encephalopathies such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle or Creutzfeldt-Jacob disease (CJD) and Gerstmann-Straussler-Scheinker syndrome (GSS) in humans is caused by a transmissible agent designated prion. The 'protein only' hypothesis proposes that the prion consists partly or entirely of a conformational isoform of the normal host protein PrP(C), designated PrP(*)(1) and that the abnormal conformer, when introduced into the organism, causes the conversion of PrP(C) into a likeness of itself. PrP(*) may be congruent with PrP(Sc), a protease-resistant, aggregated conformer of PrP that accumulates mainly in brain of almost all prion-infected organisms. PrP(C) consists of a flexible N-terminal half, comprising Cu(2+)-binding octapeptide repeats, and a globular domain consisting of three alpha-helices, one short antiparallel beta-sheet and a single disulphide bond. It is anchored at the outer cell-surface by a glycosyl phosphatidylinositol (GPI) tail and is present in almost all tissues, however, mainly in brain. Compelling linkage between the prion and PrP was established by biochemical and genetic data and led to the prediction that animals devoid of PrP should be resistant to experimental scrapie and fail to propagate infectivity. This prediction was indeed borne out, adding substantial support to the 'protein only' hypothesis. In addition, the availability of PrP knock-out mice provided an approach to carry out reverse genetics on PrP, both in regard to prion disease and to its physiological role.

124. Wells, G. A., Hawkins, S. A., Austin, A. R., Ryder, S. J., Done, S. H., Green, R. B., Dexter, I., Dawson, M., & Kimberlin, R. H. (2003). Studies of the transmissibility of the agent of bovine spongiform encephalopathy to pigs. J Gen Virol, 84(Pt 4), 1021-31.

Abstract: Studies to test the transmissibility of the bovine spongiform encephalopathy (BSE) agent to pigs began in 1989. Parenteral inoculation of the agent by three routes simultaneously (intracranially, intravenously and intraperitoneally) produced disease with an incubation period range of 69-150 weeks. Pre-clinical pathological changes were detected in two pigs killed electively at 105 and 106 weeks post-inoculation. Infectivity was detected by bioassay in inbred mice in the CNS of those pigs that developed spongiform encephalopathy. Infectivity was also found in the stomach, jejunum, distal ileum and pancreas of terminally affected pigs. These findings show that pigs are susceptible to BSE. In contrast, disease failed to occur in pigs retained for 7 years after exposure by feeding BSE-affected brain on three separate days, at 1-2 week intervals. The amounts fed each day were equivalent to the maximum daily intake of meat and bone meal in rations for pigs aged 8 weeks. No infectivity was found in tissues assayed from the pigs exposed orally. This included tissues of the alimentary tract. It is suggested that these pigs did not become infected. The relatively high oral exposure used in these experiments compared with feed-borne exposure in the field may explain the absence of an epidemic of spongiform encephalopathy in domestic pigs concurrent with the BSE epidemic in the UK.

125. Wells GAH. (2003). Pathogenesis of BSE. VETERINARY-RESEARCH-COMMUNICATIONS. SEP 2003; 27 Suppl. 1 : 25-28.

Abstract: Before the emergence of bovine spongiform encephalopathy (BSE) and recognition of its zoonotic potential, the major example of the transmissible spongiform encephalopathies (TSEs) of animals was scrapie of sheep. But there is no evidence that scrapie transmits naturally to any species other than sheep and goats. The pathogenesis of scrapie has been studied most in experimental laboratory rodent species. In most experimental models of scrapie, after peripheral non-neural routes of infection, replication of the agent can first be detected in lymphoreticular system (LRS) tissue. When the route of introduction of agent into the body is localized, initial involvement will be in LRS tissue draining the infection site. Thereafter, there is a striking amplification of the agent in the LRS and spread by lymphatic/ haematogenous routes, giving widespread dissemination in the LRS. This precedes replication in the CNS, but is not the means by which infection reaches the CNS. There is now substantial evidence from experimental models of scrapie that involvement of the CNS is by peripheral nervous system (PNS) pathways. In some models employing oral exposure the earliest localized LRS replication is in the gut-associated lymphoid tissue (GALT) and autonomic PNS routing to the CNS has been implicated. However, the relative importance of different routes of spread of TSEs within the body is determined by a number of host- and agent-dependent factors and, therefore, generalizations from an experimental model to a natural disease across a species barrier may not be appropriate. With the occurrence of BSE and recognition of its food-borne route of transmission via meat and bone meal, has come greater awareness of the probable importance of the oral route of infection in ruminant species affected by TSEs. In consequence, studies have increasingly focused on the natural host species to examine pathogenetic events.

126. Westaway, D., & Carlson, G. A. (2002). Mammalian prion proteins: enigma, variation and vaccination. Trends Biochem Sci, 27(6), 301-7.

Abstract: Although misfolding of the cellular prion protein PrP(C) into an alternative form, denoted PrP(Sc), is a key event in prion infections, the normal function of PrP(C) remains to be clearly defined. Many PrP(C)-binding proteins have been identified, but authentication of these interactions in functional assays is incomplete. Doppel (Dpl), a recently discovered PrP-like protein, might provide a new avenue by which to explore physiological and pathological functions of PrP. For example, overexpression of Dpl causes apoptotic cerebellar cell death that is abrogated by PrP(C), indicating that these two proteins can act in a common pathway. Despite our incomplete understanding of PrP(C), immunological targeting of this PrP(Sc) precursor has produced encouraging results, indicating a potential point of intervention against these fatal diseases.

127. Wilesmith, J. W., Ryan, J. B. M., Stevenson, M. A., Morris, R. S., Pfeiffer, D. U., Lin, D., Jackson, R., & Sanson, R. L. (2000). Temporal Aspects of the Epidemic of Bovine Spongiform Encephalopathy in

Great Britain: Holding-Associated Risk Factors for the Disease. Veterinary Record, 147(12), 319-325.
Abstract: The objectives of this study were first to describe the pattern of the epidemic of bovine spongiform encephalopathy (BSE) in Great Britain in terms of the temporal change in the proportion of all cattle holdings that had experienced at least one confirmed case of ass to lune 30, 1997, and secondly to identify risk factors that influenced the date of onset of a holding's first confirmed BSE case. The analyses were based on the population of British cattle at risk, derived from agricultural census data collected between 1986 and 1996, and the BSE case data collected up to lune 30, 1997 The unit of interest was the cattle holding and included all those recorded at least once on annual agricultural censuses conducted between lune 30, 1986, and lune 30, 1996. The outcome of interest was the date on which clinical signs were recorded in a holding's first confirmed case of BSE, termed the BSE onset date. Univariate and multivariate survival analysis techniques were used to describe the temporal pattern of the epidemic. The BSE epidemic in Great Britain started in November 1986, with the majority of affected holdings having their BSE onset date after February 1992. After adjusting for the effect of the size and type of holding; holdings in the south of England (specifically those in the Eastern, South east and South west regions) had 2.22 to 2.43 (95 per cent confidence interval [CI] 2.07 to 2.58) times as great a monthly hazard of having a BSE index case as holdings in Scotland. After adjusting for the effect of region and type of holding, holdings with more than 53 adult cattle had 5.91 (95 per cent CI 5.62 to 6.21) times as great a monthly hazard of having a BSE index case as holdings with seven to 21 adult cattle. Dairy holdings had 3.06 (95 per cent CI 2.96 to 3.16) times as great a monthly hazard of having a BSE index case as beef suckler holdings. These analyses show that there were different rates of onset in different regions and in holdings of different sizes and types, that the epidemic was propagated most strongly in the south of the country, and that the growth of the epidemic followed essentially the same pattern in each region of the country, with modest temporal lags between them. The control measures imposed in 1988 and 1990 brought the expansion of the epidemic under control, although the rate of progress was slowed by those regions where the effectiveness of the control methods took some time to take full effect.

128. Willerroider M. (2003). Routine tests reveal unknown strains of BSE prions. NATURE-. OCT 16 2003; 425 (6959) : 648.

129. Yamanouchi, K. (1998). [Progress of prion theory and bovine spongiform encephalopathy]. Tanpakushitsu Kakusan Koso, 43(1), 65-74.

130. Yang, C. M. (2000). Evidence for Oxidative Damage to Prion Protein in Prion Diseases. Chinese Science Bulletin, 45(17), 1546-1554.
Abstract: In prion diseases the irreversible protein structural transformation process is completed in the brains of mammals within a few months, the uniformly generated infectivity displays extraordinary resistance to inactivation, suggesting that a vital energy source is required for the production of infectious particles. Considering the high oxygen-respiration rate in the brains, prion protein oxidative damage can be the crucial factor. Both theoretical consideration of the nature of protein radical reactions and a large body of previously unraveled feature of scrapie and prion diseases have provided multiple distinct lines of compelling evidence which persuasively support a suggestion that the infectious agents may be prion (free) radicals produced from protein oxidative damage. This paper describes that scrapie prions are most likely formed from prion radicals and oxidative species-mediated sequence-specific cross-linking of benign prion proteins.

131. Yokoyama, T., Kimura, K. M., Ushiki, Y., Yamada, S., Morooka, A., Nakashiba, T., Sassa, T., & Itohara, S. (2001). In Vivo Conversion of Cellular Prion Protein to Pathogenic Isoforms, as Monitored by Conformation-Specific Antibodies. Journal of Biological Chemistry, 276(14), 11265-11271.
Abstract: The central event in prion disease is thought to be conformational conversion of the cellular isoform of prion protein (PrPC) to the insoluble isoform PrPSc. We generated polyclonal and monoclonal antibodies by immunizing PrPC-null mice with native PrPC. All seven monoclonal antibodies (mAbs) immunoprecipitated PrPC, but they immunoprecipitated PrPSc weakly or not at all, thereby indicating preferential reactivities to PrPC in solution. Immunoprecipitation using these mAbs revealed a marked loss of PrPC in brains at the terminal stage of illness. Histoblot analyses

using these polyclonal antibodies in combination of pretreatment of blots dissociated PrPC and PrPSc in situ and consistently demonstrated the decrease of PrPC at regions where PrPSc accumulated. interestingly, same mAbs showed immunohistochemical reactivities to abnormal isoforms. One group of mAbs showed reactivity to materials that accumulated in astrocytes, while the other group did so to amorphous plaques in neuropil. Epitope mapping indicated that single mAbs have reactivities to multiple epitopes, thus implying dual specificities. This suggests the importance of octarepeats as a part of PrPC-specific conformation. Our observations support the notion that loss of function of PrPC may partly underlie the pathogenesis of prion diseases. The conversion of PrPC to PrPSc may involve multiple steps at different sites.

Appendix B

BSE Patent Search

Part 1: Patent Search on BSE

Title: US6677125: Assay for disease related conformation of a protein and isolating same

Date: 2004-01-13

Inventor: **Prusiner, Stanley B.**; San Francisco, CA
Safar, Jiri G.; Walnut Creek, CA

Assignee: **The Regents of the University of California**, Oakland, CA

Abstract: An assay method is disclosed which isolates and detects the presence of a disease related conformation of a protein (e.g., PrP^{Sc}) present in a sample also containing the non-disease related conformation of the protein (e.g., PrP^{C}). The sample is treated (e.g., contacted with protease) in a manner which hydrolyzes the disease related conformation and not the non-disease related conformation. The treated sample is contacted with a binding partner (e.g., a labeled antibody which binds PrP^{Sc}) and the occurrence of binding provides and indication that PrP^{Sc} is present. Alternatively the PrP^{Sc} of the treated sample is denatured (e.g., contacted with guanadine) or unfolded. The unfolded PrP^{Sc} is contacted with a binding partner and the occurrence of binding indicates the presence of PrP^{Sc} in the sample. In another embodiment, PrP^{Sc} and PrP^{C} are reacted with a labeled antibody that binds both conformations and a conformation that binds only the disease related conformation, and the presence of the disease related conformation is determined by comparing the two.

Title: US20040025715A1: Concept for slurry separation and biogas production

Date: 2004-02-12

Inventor: **Bonde, Torben**; Egaa, Denmark
Pedersen, Lars Jorgen; Hadsten, Denmark

Assignee: None

Abstract: The present invention concerns an anaerobic digestion of animal manures, energy crops and similar organic substrates. The process is capable of refining nutrients comprised in the digested biomass to fertilizers of commercial quality. The invention also provides a method for processing animal carcasses or fractions thereof including meat and bone meal etc., with the objective of providing an alternative means for processing the organic waste material of animal origin while at the same time facilitating the production of fertilizers. The risk of spreading BSE prions or any other prions to animals or humans is thus substantially reduced if not eliminated. The biogas and slurry separation system according to the present invention is preferably integrated with the operations of animal husbandries into a total concept in which the internal and external performances of animal husbandries are optimised. The internal performances concern quality aspects related to the management of the animal houses and include industrial hygiene, animal welfare, gaseous and dust emissions and food safety. The external performances concern mainly energy production and emissions to the environment of nutrients and greenhouse gases and the sale of high quality food product.

Title: WO04007546A1: MUTANT PROTEINS AND USE THEREOF FOR THE MANUFACTURE OF MEDICAMENTS AND THE TREATMENT OF HUMANS OR ANIMALS SUFFERING FROM CONFORMATIONAL DISEASES

Date: 2004-01-22

Inventor: **ZAHN, Ralph**; Michelstr. 13, CH-8049 Zürich, Switzerland

Assignee: **EIDGENOESSISCHE TECHNISCHE HOCHSCHULE ZUERICH**, Rämistrasse 101, CH-8092 Zürich, Switzerland

Abstract: The invention relates to a mutant prion protein (PrP), the globular domain of which comprises an engineered second disulfide bond in a similar position as in the human doppel protein (hDpl). In an embodiment, the prion protein has an engineered extra disulfide bond in the presumed 'factor X' binding epitope and is accommodated with slight, strictly localized conformational changes to inhibit prion propagation in human and animals. Also disclosed is the use of a mutant prion protein (PrP), the globular domain of which comprises at least one engineered additional disulfide bond in a similar position as in the human doppel protein, or fragments thereof for therapeutic treatment or for the manufacture of a medicament for therapeutic treatment of proteins causing disease after a conformational transition, e.g. Transmissible Spongiform Encephalopathy (TSE), variant forms of

Creutzfeldt-Jakob disease (CJD), fatal familial insomnia (FFI), and Gerstmann-Sträussler-Scheinker syndrome (GSS) in human. Further, the use of the PrP mutant protein for in vivo generation of disulfide mutants of prion proteins or fragments thereof is carried out in order to enable an intended ther-apy of TSE in animals, e.g. by somatic gene therapy with lentiviral vector, where TSE includes bovine spongiform encephalopathy (BSE), scrapie in sheep, feline spongiform encephalopathy (FSE), and chronic wasting disease (CWD) in elk and deer.

Title: WO0224652A1: COMPOUNDS AND METHODS FOR DIAGNOSING AND TREATING AMYLOID-RELATED CONDITIONS

Date: 2002-03-28

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Abstract: The invention provides methods for diagnosing and treating amyloid-related conditions and compounds useful for the same. The invention provides for detecting, imaging, monitoring, diagnosing, and treating conditions characterized by the binding or aggregation of amyloid fibrils. More particularly, the invention relates to using quinolinehydrazone compounds for diagnosing and treating amyloidotic conditions and also as an antioxidant.

Title: WO04005930A2: DIAGNOSTIC USE OF PHARMACEUTICAL SUBSTANCES AND METHOD FOR THE DIAGNOSIS OF HUMAN AND ANIMAL NEURODEGENERATIVE DISEASES

Date: 2004-01-15

Inventor: **WILHELM, Michael**; Universitätsring 10, 06108 Halle, Germany
AGUEEV, Vladimir; Fritz-Reuter Str. 6, 06114 Halle, Germany

Assignee: **MERDIQUANT GMBH**, Weinbergweg 22, 06120 Halle/S., Germany

Abstract: The invention relates to the use of known pharmaceutical substances chloropromazine, ethacrine, quinacrine, lovastatine, doxorubicine and D-penicillamine for the diagnosis of human and animal neurodegenerative diseases such prion diseases as TSE diseases suc has BSE, CJD, vCJD, scrapie and other neurodegenerative diseases based on the formation of neurotoxic amyloid plaques. Body fluids such as whole blood, plasma, serum, liquor, urine, milk, lymph, lymph plasma and saliva, i.e. body fluids containing proteins, are used as test media. Previously, said substances were used therapeutically as psychopharmaceuticals, cycostatics, lipid reducres, antirheumatics and antiparasite agents. The invention also relates to a method for the diagnosis of human and animal neurodegenerative diseases, wherein said pharmaceutical substances are used.

Title: GB2390291A1: Method for producing specialty plant bio-feed from the animal mbms using edible mushroom strains, and the specialty bio-feed produced thereby

Date: 2004-01-07

Inventor: **SUK-KU * SIM**; Republic of Korea
EUN-KWAN * LEE; Republic of Korea

Assignee: **ENVIRONMENT & ENERGY TECHNOLOGY** Republic of Korea

Abstract: The present invention relates to a method for producing speciality plant bio-feed abundant in mushroom hyphae from the animal MBMs, and to the specialty bio-feed produced thereby. It is the object of the invention to solve the problem of animal feed using the animal MBMs for ruminants that is assumed to be a cause of the Bovine Spongiform Encephalopathy (BSE) that causes the people in the world as well as in Europe in a panic. In accordance with the invention, the specialty plant bio-feed abundant in mushroom hyphae is not only safe from the BSE, but also is rich in organic and inorganic matters, enhances the digestion efficiency and creates high added values with the rich anti-cancer ingredients and new functional matters.

Title: US20030229910A1: Short fragment homologous replacement to provide BSE resistant cattle

Date: **2003-12-11**

Inventor: **Metz, Richard**; Lawrenceville, NJ, United States of America
Blaese, R. Michael; New Hope, PA, United States of America

Assignee: None

Abstract: A method for generating cattle resistant to Bovine Spongioform Encephalopathy through targeted alterations in the PrP gene is disclosed. The PrP gene of a cultured cells is altered to prevent its translation or to encode a dominant disease-resistant form of the protein, and the nucleus of the altered cell is used to clone a founder animal. In one embodiment, a single-stranded DNA fragment containing the alteration is used in single-stranded short fragment homologous replacement to alter the PrP gene.

Derwent Title: Agents for combating beta-amyloid plaque deposition, specifically new monomeric or oligomeric heterocyclic bis-amides and amino-ketones, useful for treating Alzheimer's disease and prion diseases

Date: **2003-12-04**

Inventor: **Schrader, Thomas, Prof. Dr.**; Mettmann, Germany 40822
Riesner, Detlev, Prof. Dr.; Düsseldorf, Germany 40547
Rzepecki, Petra; Marburg, Germany 35037
Nagel-Steger, Luitgard, Dr.; Langenfeld, Germany 40764
Molt, Oliver; Erkrath, Germany 40699
Zadmard, Reza; Gießen, Germany 35392
Wehner, Mark; Düsseldorf, Germany 40477
Kirsten, Christian, Dr.; Burscheid, Germany 51399
Aschermann, Katja, Dr.; Wuppertal, Germany 42111

Assignee: **TransMIT Gesellschaft für Technologietransfer mbH**, Gießen, Germany35394

Title: US6657105: Prion gene modified mouse which exhibits heart anomalies Date: 2003-12-02

Inventor: **Itohara, Shigeyoshi**; Saitama, Japan
Onodera, Takashi; Ibaraki, Japan
Tsubone, Hirokazu; Ibaraki, Japan

Assignee: **Riken** Japan

Abstract: The present invention relates to a method for detecting an aberrant animal-derived prion gene wherein the method comprises steps of introducing a prion gene of an animal into a mouse to produce a prion gene modified mouse and determining that the prion gene is aberrant when the prion gene modified mouse exhibits heart anomalies; a prion gene modified mouse which exhibits heart anomalies; and a method for detecting drugs which reduce abnormal waves in an electrocardiogram of the mouse.

Title: WO0239826A1: DECONTAMINATION OF ANIMAL FEED CONTAINING PRION (eg. BSE agent).

Date: **2002-05-23**

Inventor: **KEMP, PHILIP WILLIAM**; Carnarvon, Derreen Road, Warwick, Queensland 4370, Australia

Assignee: **AUSTECH STERILE RESOURCE RECOVERY PTY LTD**, 6 Bell Street, Toowoomba, Queensland 4350, Australia

Abstract: A method of producing an animal feed that is free from transmittable degenerativeencephalopathies is provided. Central to the method is alkali treatment of animalmaterial at a pH of at least 8.5 under temperature conditions below 100 C at atmospheric pressure. This method provides a decontaminated animal feed produced under relatively low temperature and pressure conditions that are achievable instandard animal carcass rendering facilities.

Title: WO03095429A1: ACTIVE SUBSTANCES FOR THE TREATMENT, DIAGNOSIS AND PROPHYLAXIS OF DISEASES IN WHICH ABNORMAL PROTEIN STRUCTURES OCCUR

Date: 2003-11-20**Inventor:** **SCHRADER, Thomas**; Schwalbenweg 11, 40822 Mettmann, Germany
RIESNER, Detlev; Sonsbecker Str. 19, 40547 Düsseldorf, Germany
RZEPECKI, Petra; Wettergasse 41, 35037 Marburg, Germany
NAGEL-STEGER, Luitgard; Alt-Langenfeld 12, 40764 Langenfeld, Germany
WEHNER, Mark; Lenzhahner Weg 1A, 65527 Niedernhausen, Germany
KIRSTEN, Christian; Grosshamberg 11e, 51399 Burscheid, Germany
MOLT, Oliver; Neuenhausstr. 16, 40699 Erkrath, Germany
ZADMARD, Reza; Unterhof 69, App. 1013, 35392 Giessen, Iran
ASCHERMANN, Katja; Ilexweg 1a, 42111 Wuppertal, Germany

Assignee: **TRANSMIT GESELLSCHAFT FÜR TECHNOLOGIETRANSFER MBH**, Kerkrader Str. 3, 35394 Giessen, Germany

Abstract: The invention concerns diseases in which abnormal protein structures occur. This includes, for example, Alzheimer's disease, Creutzfeldt-Jakob disease, BSE and other prion diseases. The aim of the invention is to provide novel active substances, which prevent the formation of amyloid plaques and decompose already existing plaques and which can be used for the treatment, diagnosis and prophylaxis of diseases involving abnormal protein structures. This is accomplished by the provision of heterocyclic or aromatic active substances having a rigid structure and a donor-acceptor-donor (DAD) pattern on hydrogen bridge donors and acceptors, which correspond to the ß-pleated sheet structure of the protein or peptide and thereby fit. The heterocyclic or aromatic active substances exist at least in the form of dimers. They identify peptides and proteins having a ß-pleated sheet structure, form stable complexes therewith, and prevent the aggregation thereof into ß-amyloid plaques. In addition, they decompose already existing ß-amyloid plaques. The invention also aims to provide synthesis methods for producing the heterocyclic or aromatic active substances.

Derwent Title (Original title in German): DE10219298A1: New chicken antibodies against prion proteins, useful for diagnosis of transmissible, specifically bovine, spongiform encephalopathy

Date: 2003-11-06

Inventor: **Lusky, Klaus, Prof. Dr.**; Biesenthal, Germany 16359
Doberschütz, Klaus-Dieter, Dr.; Berlin, Germany 12555
Henklein, Peter, Dr.; Berlin, Germany 10365
Schade, Rüdiger, Dr.; Berlin, Germany 12623
Fischer, Lothar, Dr.med. vet.; Berlin, Germany 12679
Sasse, Mirko, Dr.; Fürstenwalde, Germany 15517

Assignee: **Universitätsklinikum Charité Medizinische Fakultät der Humboldt-Universität Akademische Verwaltung - Forschung, 10117 Berlin, DE**
Institut für Veterinär-Pharmakologie und Toxikologie GmbH, Bernau, Germany16321

Abstract:

Derwent Title (original title in German): Removal of the spinal cord, from slaughtered carcasses, uses two circular saws to attack the spinal column from within and outside the carcass, to open up the spinal channel to expose the spinal cord and tissue for suction extraction

Date: 2003-11-06

Inventor: **Arbter, Conrad**; Saal, Germany 97633

Assignee: **Arbter GmbH Entwicklung, Patente und Verwertung**, Saal, Germany97633

Abstract:

Title: NZ0512335A: Antibodies to a peptide component of myelin and or neurofilaments in a diagnostic kit

Date: **2003-10-31**

Inventor: **EBRINGER, ALAN**;

Assignee: **KING'S COLLEGE LONDON**

Abstract: Described is a method for diagnosing spongiform disease or demyelinating disease in vertebrates, including BSE, MS and CJD, which comprises assaying a biological sample for antibodies which bind to myelin and/or myelin neurofilaments or to one or more antigenic (immunogenic) parts thereof. This assay involves a test where antibodies to two brain antigens can be determined in bovine or human sera. Any reading in excess of 2 standard deviations of the healthy controls would indicate a positive response. Furthermore, the test should be positive (above 2 standard deviations) for both antigens: (A) Bovine myelin protein and (B) Bovine neurofilaments. This is the first assay that describes measurements of autoantibodies to brain antigens in BSE affected cattle and patients with MS and CJD, the results of which are shown for BSE in the accompanying Figure.

Title: WO03089609A2: SHORT FRAGMENT HOMOLOGOUS REPLACEMENT TO PROVIDE BSE RESISTANT CATTLE

Date: **2003-10-30**

Inventor: None

Assignee: **METZ, Richard**, 37 Winthrop Rd., Lawrenceville, NJ 08648, United States of America
BLAESE, Michael, R., 48 Woodside Lane, New Hope, PA 18938, United States of America

Abstract: A method for generating cattle resistant to Bovine Spongioform Encephalopathy through targeted alteration in the PrP gene is disclosed. The PrP gene of a cultured cell is altered to prevent its translation or to encode a dominant disease-resistant form of the protein, and the nucleus of the altered cell is used to clone a founder animal. In one embodiment, a single-stranded DNA fragment containing the alteration is used in single-stranded short fragment homologous replacement to alter the PrP gene.

Title: EP1354517A1: Device and method for removing BSE contaminated material

Derwent title: Cutting system, to remove spinal cord from animal carcass, has hollow conical body with circular blade, and suction channel to take in loosened material as it is cut until finally severed and extracted

Date: **2003-10-22**

Inventor: **Kreis, Armin**;

Assignee: **BVS Beratung-Verkauf-Service für die Fleischwirtschaft GmbH**

Abstract:

Title: EP0929813B1: DIAGNOSIS OF SPONGIFORM DISEASE

Date: **2003-10-08**

Inventor: **EBRINGER, Alan**;

Assignee: **KING'S COLLEGE LONDON**

Abstract: A diagnostic test is provided for spongiform encephalopathy and other demyelinating conditions in mammals which comprises assaying antibodies present in the mammal which bind to an antigenic peptide which exhibits molecular mimicry of a mammalian myelin peptide, e.g. one having the sequence FSWGAEGQK. This test is useful for detecting BSE in cattle by assaying sera collected from the cattle for antibodies to a species of Acinetobacter, Agrobacterium or Ruminococcus, or a peptide having a sequence present in said species which mimics a peptide of bovine myelin and identifying animals having a level of antibodies at least about two standard deviations above that of healthy control animals.

Title: WO03036303C1: TECHNIQUE DE DIAGNOSTIC
Date: 2003-09-18
Inventor: STACK, MICHAEL, JAMES; United Kingdom
CHAPLIN, MELANIE, JANE; United Kingdom
CLARK, JEMMA; United Kingdom
Assignee: THE SECRETARY OF STATE FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS United Kingdom
STACK, MICHAEL, JAMES United Kingdom
CHAPLIN, MELANIE, JANE United Kingdom
CLARK, JEMMA United Kingdom

Abstract: A method for typing a strain of a transmissible spongiform encephalophathy (TSE) in an infected animal, said method comprising: a) separating a sample of abnormal prion protein on the basis of molecular weight and/or glycoform ratios, and detecting the separated forms; b) detecting in the sample the presence of a peptide sequence, wherein the presence of said peptide sequence within abnormal prion protein is capable of distinguishing a particular strain of TSE from others, and c) using the results of (a) and (b) to determine the type of TSE strain present in the sample. The method may be used in particular to distinguish BSE from scrapie in sheep.

Title: US6605445: Rapid method of determining clearance of prion protein
Date: 2003-08-12
Inventor: Petteway, Steve R.; Cary, NC
Lee, Douglas C.; Apex, NC
Kozak, Robert W.; Foster City, CA
Assignee: Bayer Corporation, Pittsburgh, PA

Abstract: Transmissible spongiform encephalopathies (TSEs) are a class of fatal, neurodegenerative diseases found in many mammalian species. Human TSEs include kuru, Creutzfeldt-Jakob disease (CJD), and fatal familial insomnia. Non-human TSEs include sheep scrapie, bovine spongiform encephalopathy (BSE), feline spongiform encephalopathy, and chronic wasting diseases in elk and mule deer. These neurodegenerative diseases are caused by prions and display characteristic amyloid plaque deposition. The only known component of the infectious prion is an abnormal, disease-causing isoform of the prion protein (PrP), called PrP scrapie (PrP^{Sc}). During a post-translational process, PrP^{Sc} is formed from the normal, cellular PrP isoform (PrP^{C}). The scrapie isoform is less soluble, more proteinase-resistant, and more susceptible to aggregation than the wildtype protein. The claimed invention is directed toward a rapid, specific, sensitive, and quantitative method for the detection of TSE clearance from a plasma product utilizing a chemiluminescent Western blot immunoassay. This immunoassay format can detect picogram concentrations of PrP^{RES} (proteinase resistant PrP fragment) and is linear over a 3-5 log range. The invention is useful for tracking the clearance of PrP^{Sc} during plasma manufacturing processes.

Title: JP2003164253A2: METHOD FOR DRESSING SLAUGHTERED LIVESTOCK
Date: 2003-06-10
Inventor: TAKENOUCHI ATSUSHI;
KIKUCHI REIICHI;
OE TADAO;
Assignee: TOYO NETSU KOGYO KK
TPG KK

Abstract: PROBLEM TO BE SOLVED: To provide a method for dressing slaughtered livestock for dealing with the animal in the spine thereof in an entirely blocked condition from the outside without cutting off or exposing the spine cord in the spine.

SOLUTION: This method for dressing slaughtered livestock, involving a BSE (bovine spongeform encephalopathy) examination, comprises carrying out the BSE examination by extracting a specimen from a cattle head and disposing of the spine cord by incineration; more specifically, this method includes the step of separating both sides of the spine 1 of a cattle body by cutting off both the sides along the spine 1 along cutting lines 6 and 8 and then disposing the spine 1 together with the spine cord 4 thereinside. Thus, the spine cord can be prevented from scattering around and sticking onto the surrounding meat part or operators, cutting tools and the like therearound, the spine cord as a hazardous site of BSE can entirely be disposed of by incineration, therefore leading to higher safety against BSE.

Title: JP2003144042A2: METHOD AND APPARATUS FOR REMOVING SPINAL CORD IN TREATING BEEF

Date: 2003-05-20

Inventor: MURAKAMI YUKIHARU;

Assignee: MURAKAMI SHOTEN:KK

Abstract: PROBLEM TO BE SOLVED: To provide a method and a related apparatus for removing the spinal cord of a cow in treating beef capable of surely removing the spinal cord as a specific hazardous site for BSE at low cost in a short time, thus preventing the beef from contamination in dismantling the cow.

SOLUTION: This method for removing the spinal cord of a cow in treating beef through halving the slaughtered cow and cutting it to carcass comprises the following practice: prior to the halving operation after excising the head, an air tube 6 is inserted from the cervical vertebrae 8 side into the spinal hole and injected with compressed air from its feed source to discharge the spinal cord as the specific hazardous site for BSE via the cervical vertebrae 8 side by the aid of the injection force of the compressed air.

Title: WO03036303A1: DIAGNOSTIC METHOD

Date: 2003-05-01

Inventor: **STACK, Michael, James**; Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

CHAPLIN, Melanie, Jane; Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

CLARK, Jemma; Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

Assignee: **THE SECRETARY OF STATE FOR ENVIRONMENT, FOOD AND RURAL AFFAIRS**, (DEFRA) Nobel House, 17 Smith Square, London SW1P 3JR, acting through the Veterinary Laboratories, Agency of New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

Abstract: A method for typing a strain of a transmissible spongiform encephalophathy (TSE) in an infected animal, said method comprising: a) separating a sample of abnormal prion protein on the basis of molecular weight and/or glycoform ratios, and detecting the separated forms; b) detecting in the sample the presence of a peptide sequence, wherein the presence of said peptide sequence within abnormal prion protein is capable of distinguishing a particular strain of TSE from others, and c) using the results of (a) and (b) to determine the type of TSE strain present in the sample. The method may be used in particular to distinguish BSE from scrapie in sheep.

Title: GB2379737A: DIAGNOSTIC METHOD FOR SPONGIFORM ENCEPHALOPATHY DISEASE

Date: 2003-03-19

Inventor: **ELISABETH * GUILLAUME**; France

DENIS FRANCOIS * HOCHSTRASSER; Switzerland

JEAN-CHARLES * SANCHEZ; Switzerland

Assignee: **UNIVERSITE DE GENEVE** Switzerland

Abstract: Diseases such as BSE, CJD or scrapie are diagnosed in a subject by analysing a sample of body fluid by mass spectrometry to determine the presence of a polypeptide marker of molecular weight 3500 to 30000 where the polypeptide is present in different amounts in the body fluid of infected and non-infected subjects. The test sample may be adsorbed on a probe carrying an adsorbent material capable of binding the polypeptide. The material is e.g. a metal chelating group complexed with a metal ion. Kits for carrying out the diagnosis are also disclosed. The body fluid sample may be from humans or cattle.

Title: US20030032032A1: Early pre-symptomatic prion diagnostic blood test for encephalopathies

Date: 2003-02-13

Inventor: **Resink, Annelies**; Paris, France
Fuentes, Nathalie; Kremlin Bicetre, France
Schweighoffer, Fabien; Vincennes, France

Assignee: None

Abstract: This invention relates to compositions and methods of detecting encephalopathies in a subject. This invention also relates to genetic markers, nucleic acid preparations or libraries, and kits for use in the implementation of said detection methods. The compositions and methods of this invention can also be used for the diagnosis, characterization, progression monitoring, etc. of encephalopathies, including at early stages thereof, particularly Transmissible Spongiform Encephalopathies (TSE), including Bovine Spongiform Encephalopathies (BSE, "Mad Cow disease").

Title: US6517855: Method of sterilizing

Date: 2003-02-11

Inventor: **Prusiner, Stanley B.**; San Francisco, CA
Supattapone, Surachai; San Francisco, CA
Scott, Michael R.; San Francisco, CA

Assignee: **The Regents of the University of California**, Oakland, CA

Abstract: A method of sterilizing objects as well as the sterilized objects obtained from the method are disclosed. The method involves contacting an object such as a medical device to be reused with polycationic dendrimer under conditions which result in rendering a conformationally altered protein (e.g. a prion) non-infectious. A disinfecting agent or surgical scrub composition which comprises the dendrimers is also disclosed as are gelatin capsules treated with polycationic dendrimers.

Title: DE10132444A1: Treating or preventing bovine spongiform encephalopathy or related diseases, by administration of thiocyanate ions, preferably as nutritional supplement or feed additive

Derwent title: Treating or preventing bovine spongiform encephalopathy or related diseases, by administration of thiocyanate ions, preferably as nutritional supplement or feed additive

Date: 2003-01-30

Inventor: **Weuffen, Wolfgang, Prof. Dr.med.habil. Dr.rer.nat.**; 17498 Guest, Germany

Assignee: **Weuffen, Wolfgang, Prof. Dr.med.habil. Dr.rer.nat.**, Guest, Germany17498

Abstract:

Derwent Title: DE10113151A1: Composition useful for treating bovine spongiform encephalopathy or Creutzfeld-Jakob disease, comprising farina animale carbonata

Date: 2003-01-30

Inventor: **Alagna-Megal, Angelika**; 13357 Berlin, Germany

Assignee: **Alagna-Megal, Angelika**, Berlin, Germany13357

Title: DE10133159A1: Corpuscles in contaminated food enter the blood stream and cause repeated microembolisms in brain and cause bovine spongiform encephalopathy

Date: 2003-01-23

Inventor: **Schubert, Werner, Dr.med.**; 45468 Mülheim, Germany

Assignee: **Schubert, Werner, Dr.med.**, Mülheim, Germany45468

Abstract:

Title: WO03000714A3: COMPOSITIONS AND METHODS FOR PREVENTING PROTEIN AGGREGATION IN NEURODEGENERATIVE DISEASES

Date: 2003-01-03

Inventor: **GHANBARI, Hossein, A.**; 9460 Tobin Circle, Potomac, MD 20854, United States of America
GHANBARI, Kasra; 9460 Tobin Circle, Potomac, MD 20854, United States of America

Assignee: **PANACEA PHARMACEUTICALS, INC.**, 9700 Great Seneca Hwy., Rockville, MD 20850, United States of America

Abstract: Disclosed are methods for treating a disease that involves protein aggregation, including Alzheimer's, Parkinson's, prion diseases such as BSE and CJD, and Down's syndrome. The methods involve administering to a subject suspected of having the disease a very high affinity antibody fragment immunoreactive with the protein that is aggregating. Such treatment will have the effect of preventing, slowing, or halting the disease progression by inhibiting protein aggregation

Title: US20020194635A1: Transgenic animals resistant to transmissible spongiform encephalopathies

Date: 2002-12-19

Inventor: **Dunne, Patrick W.**; La Grange, TX, United States of America
Piedrahita, Jorge; College Station, TX, United States of America

Assignee: None

Abstract: The invention provides modified prion-encoding genes for the creation of transgenic bovine and cervid animals resistant to transmissible spongiform encephalopathies including bovine spongiform encephalopathy (BSE). The transgenic animals homozygous for the mutant genes continue to express a functional copy of the prion-encoding gene, thereby not interfering with the normal role of the polypeptide and effectively decreasing tendency for alteration of sleep-wake cycles.

Part 2: Patent Search on Prion Proteins (PrP-cellular) and Prions (PrP-scrapie)

WO03096023A1: SARCOSYL BUFFER AND ITS USE FOR THE PURIFICATION OF PRION PROTEIN
Date: 2003-11-20
Abstract:
The invention is in the field of prion protein (PrP). The invention relates to compositions and methods for selectively enhancing destruction of PrPc over PrPsc. In particular the invention relates to a composition comprising an ionic surfactant/detergent such as N-lauroyl sarcosine, a non-ionic surfactant/detergent such as Triton X-100, optionally a buffering agent such as Sodium Acetate at acidic pH such as pH 4.5, and optionally a reducing agent such as Dithiothreitol (DTT), and to methods of using such a composition. In particular, the invention relates to a composition comprising 10% w/v N-lauroyl sarcosine, 11% w/v Triton X-100, IM Dithiothreitol (DTT) and 40mM Sodium Acetate pH 4.5.
Inventors:
JACKSON, Graham, Stuart; MRC Prion Unit, Institute of Neurology, National Hospital, Queen Square, London WC1 3BG, United Kingdom
COLLINGE, John; MRC Prion Unit, Institute of Neurology, National Hospital, Queen Square, London WC1N 3BG, United Kingdom
WADSWORTH, Jonathan, D., F.; MRC Prion Unit, Institute of Neurology, National Hospital, Queens Square, London WC1 3BG, United Kingdom
CLARKE, Anthony, Russel; Department of Biochemistry, University of Bristol,School of Medical Science, University Walk, Clifton, Bristol BS8 1TD, United Kingdom
HAWKE, Simon; Department of Neurogenetic, Imperial College, Norfolk Place, London W2 1PG, United Kingdom
Assignee
MEDICAL RESEARCH COUNCIL, 20 Park Crescent, London W1B 1AL, United Kingdom

WO03093505A2: METHOD FOR DETERMINING THE PRESENCE OF AN UNCONVENTIONAL TRANSMISSIBLE AGENT RESPONSIBLE FOR TRANSMISSIBLE SUBACUTE SPONGIFORM ENCEPHALOPATHY
Date: 2003-11-13
Inventor
MOUTHON, Franck; 22, rue de l'Amiral Mouchez, F-75014 Paris, France
NOUVEL, Virginie; 8, boulevard Morland, F-75004 Paris, France
DESLYS, Jean-Philippe; 5, rue de la Ferme, F-78150 Le Chesnay, France
Assignee:
COMMISSARIAT A L'ENERGIE ATOMIQUE, 31-33, rue de la Fédération, F-75015 Paris, France
Abstract:
The invention concerns a method for identifying genes whereof the cellular expression is correlated to the presence of an unconventional transmissible agent, or a pathological form of a prion protein (PrP). The invention further concerns a method for determining the presence of an unconventional transmissible agent responsible for transmissible subacute spongiform

encephalopathy in a biological sample as well as a method for selecting a compound capable of slowing down, stopping or preventing the development of a transmissible subacute spongiform encephalopathy. Finally, the invention concerns primers, nucleotide probes and kits for implementing the method for determining the presence of an unconventional transmissible agent.

Title: EP1343391A1: METHOD FOR MODIFYING THE PROTEIN STRUCTURE OF PRIONS PRP?SC IN A TARGETED MANNER
Date: 2003-09-17
Inventor
KORTSCHACK, Fritz;
HEINZ, Volker;
Assignee
Kortschack, Fritz
Abstract
None

Title: US6620629: Method for detecting prions
Date: 2003-09-16
Inventor:
Prusiner, Stanley B.; San Francisco, CA
Safar, Jiri; Concord, CA
Assignee: The Regents of the University of California, Oakland, CA
Abstract: The present invention provides assays for identifying the levels of both protease sensitive and protease resistant conformers of PrP^{Sc} in a sample. In a preferred embodiment, the assay comprises determining levels of total PrP^{Sc} in a sample, subjecting the PrP^{Sc} fraction to treatment with a protease that selectively hydrolyzes the protease sensitive PrP^{Sc} ($sPrP^{Sc}$) conformers, and quantifying the levels of $sPrP^{Sc}$ in the sample. The ability to detect $sPrP^{Sc}$ allows early detection of prions, since the PrP^{Sc} in easily accessible biological samples such as blood is predominantly $sPrP^{Sc}$. The ratio of $sPrP^{Sc}$ to $rPrP^{Sc}$ also allows the identification of a particular prion strain in an infected sample.

Title: US6617119: Assay for specific strains of multiple disease related conformations of a protein
Published/Filed: 2003-09-09
Inventor: Prusiner, Stanley B.; San Francisco, CA
Safar, Jiri G.; Concord, CA
Cohen, Fred E.; San Francisco, CA
Assignee: The Regents of the University of California, Oakland, CA
Abstract: Assay methodology of the invention allows for: (1) determining if a sample contains a conformation of a protein which is associated with disease and the concentration and amount of such if present; (2) determining the amount of protease resistant disease related protein in a sample and by subtracting that amount from the total amount of disease related protein present determining the amount of protease sensitive disease protein in the sample; and (3) determining the strain and incubation time of a disease related protein by (i) relating the relative amounts of protease resistant and protease sensitive protein to known strains to thereby determine the strain; and (ii) plotting the concentration of protease sensitive protein on a graph of incubation time versus concentration of protease sensitive protein for known strains to predict the incubation time of an unknown strain of pathogenic protein in a sample.

Title: WO03059386A2: PRION PROTEIN CARRIER-CONJUGATES
Date: 2003-07-24
Inventor: BACHMANN, Martin; Goldackerweg 8, CH-8472 Seuzach, Switzerland
MAURER, Patrick; Richenbergstrasse 38, CH-8400 Winterthur, Germany
PELLICIOLI, Erica; Seehaldenweg 17, CH-8804 Au, Switzerland
RENNER, Wolfgang, A; Grenzsteig 9, CH-8802 Kilchberg, Switzerland
Assignee: CYTOS BIOTECHNOLOGY AG, Wagistrasse 25, CH-8952 Zürich-Schlieren, Switzerland
Abstract: The present invention is related to the fields of molecular biology, virology, immunology and medicine. The invention provides a composition comprising an ordered and repetitive antigen or antigenic determinant array, and in particular a prion peptide or prion protein-VLP-array. More specifically, the invention provides a composition comprising a virus-like particle and at least one prion protein PrP or a dimer thereof, or a PrP peptide bound thereto. The invention also provides a process for producing the conjugates and the ordered and repetitive arrays, respectively. The compositions of the invention are useful in the production of vaccines for the treatment of prion diseases and as a pharmaccine to prevent or cure prion diseases and to efficiently induce immune responses, in particular antibody responses. Furthermore, the compositions of the invention are particularly useful to efficiently induce self-specific immune responses within the indicated context.

Title: WO03050139A3: PRION INHIBITING PEPTIDES AND DERIVATIVES THEREOF
Date: 2003-06-19
Inventor: ADESSI, Céline; 8, rue du Jura, F-74100 Ambilly, France
HALAZY, Serge; 20, chemin du Grand-Cras, F-74100 Vetraz-Monthoux, Belgium
SABORIO, Gabriela; 28, chemin de la Planche Brûlée, F-01210 Ferney-Voltaire, Mexico
SOTO-JARA, Claudio; 11 Chemin des Cherpines, CH-1228 Plan-les-Ouates, Switzerland
Assignee: SERONO INTERNATIONAL S.A. INTELLECTUAL PROPERTY ; IP Department, 12, Chemin des Aulx, CH-1228 Plan-les-Ouates Switzerland
Abstract: Short peptides and derivatives or analogs thereof for the treatment or prevention of transmissible spongiform encephalopathies, in particular CJD are herein described. These peptides and/or their derivatives have been designed to block the conformational changes that occur in the prion protein (PrP) and which are implicated in the pathogenesis of transmissible spongiform encephalopathies as well as to dissolve the fibrillar deposits already formed

Title: WO03025181A3: DNA SEQUENCES THAT ENCODE VARIABLE REGIONS OF ANTIBODIES THAT BIND TO THE PRION PROTEIN (PRP), AND PRP-BINDING POLYPEPTIDES
Date: 2003-03-27
Inventor: MOSER, Markus; Sonnenbergstr. 28, CH-8610 Uster, Switzerland
OESCH, Bruno; Haldenstrasse 13, CH-5233 Stilli, Switzerland
Assignee: PRIONICS AG, Wagistrasse 27a, CH-8952 Schlieren, Switzerland
Abstract: The invention relates to a DNA sequence that encodes variable regions of antibodies that bind to PrP. Said DNA sequence contains at least one sequence indicated under SEQ1 to SEQ4 or at least a part of said sequence, and encodes polypeptides that bind PrP. The invention further relates to a polypeptide derived from the DNA sequence, which comprises at least one

PrP-binding region, except for the polypeptides that are identical to a chain of the antibodies 6H4 or 15B3, and to the use of the polypeptides in the therapy, diagnosis and prevention of prion-related diseases.

Title: US6534036: Biological materials and methods useful in the diagnosis and treatment of diseases
Date: 2003-03-18
Inventor: Collinge, John; London, United Kingdom
Clarke, Anthony R.; London, United Kingdom
Jackson, Graham S.; London, United Kingdom
Assignee: D. Gen Limited, London, United Kingdom
Abstract: The present invention relates to a method of making a ß-form of a prion protein which preferably has more ß-sheet than .alpha.-helix structure and is soluble in the absence of a denaturant and/or is non-aggregated and exhibits partial resistance to digestion with proteinase K. The invention also relates to use of the ß-form in medicine, especially for raising antibodies useful in the treatment and/or diagnosis of prion diseases. The invention also relates to methods of screening for compounds which are capable of inhibiting and/or reversing the conversion of the native .alpha.-form of a prion protein to a ß-form, and to uses of identified compounds in medicine.

Title: WO03005037A1: METHOD FOR SEPARATING AND/OR DETECTING AND/OR IDENTIFYING AND/OR QUANTIFYING PRION PROTEINS
Date: 2003-01-16
Inventor: STEFAS, Elie; 94, allée des Fauvettes, F-34280 La Grande-Motte, France
Assignee: APOH TECHNOLOGIES SA, 911, avenue Agropolis, F-34000 Montpellier, France
Abstract: The invention concerns a method for separating and/or detecting and/or identifying and/or quantifying in a biological material at least a prion protein (PrP), characterised in that it comprises a step which consists in separating and/or detecting and/or identifying and/or quantifying a (PrP/ß2GPI) complex consisting of at least a prion protein bound to at least a form of ß2GPI.

Title: WO02088748A1: METHOD FOR THE DIAGNOSIS OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES
Date: 2002-11-07
Inventor: None listed
Assignee: SEILER, Jürgen, Ulmenstrasse 1, 37124 Rosdorf, Germany
MESSER, Horst, Hambergstrasse 10a, 37124 Rosdorf, Germany
Abstract: The invention relates to a method for the diagnosis of transmissible spongiform encephalopathies by detecting in vitro immune reactions between known antibodies and an antigen of a prion protein, wherein the prion protein (PrP) are enzymatically broken down in a segment mixture during a first step and the antibodies separated from one another are exposed to the segment mixture so that the antibodies can be assigned to the different segments of the segment mixture by measuring the immune reaction complexes. In a second step, the prion protein (PrP) of a sample to be examined is separated into a normal benign type (Prpb) and a pathological malign type (Prpm).

Title: WO0249460A1: METHOD FOR MODIFYING THE PROTEIN STRUCTURE OF PRIONS PRP^{SC} IN A TARGETED MANNER
Date: 2002-06-27
Inventor: HEINZ, Volker; Földerichstrasse 63, 13595 Berlin, Germany
Assignee: KORTSCHACK, Fritz, Katzwanger Steig 36 a, 14089 Berlin, Germany
Abstract: The invention relates to a method for modifying the protein structure of prions PrPSC in a targeted manner, in animal raw products for consumption or further processing, especially meat, subcutaneous apidose tissue, connective tissue, brain and bone, and/or for sterilising abattoir equipment, operating instruments or similar objects contaminated with infectious PrPSC, the raw products or equipment being subjected to a high pressure treatment acting on all sides, by means of a transmission medium.

Title: WO0249662A3: USE OF THE PRION PROTEIN (PRP) FOR SUPPRESSION OF BAX-MEDIATED APOPTOSIS
Date: 2002-06-27
Inventor: LEBLANC, Andrea; 1253 Marthe Mongrain, Chambly, Quebec J3L 5N4, Canada
BOUNHAR, Younes; 5014 Domal, Montreal, Quebec H3W 1W2, Morocco
ZHANG, Yan; Apartment 22, 3330 Edouard Montpetit, Montreal, Quebec H3T 1K5, China
Assignee: MCGILL UNIVERSITY, 3550 University Street, Montreal, Quebec H3A 2A7, Canada
Abstract: The present invention relates to a method of protecting neurons against bax-mediated apoptosis and assays to identify agents which modulate neuron apoptosis. The present invention further relates to apoptosis modulation in other tissues in which prion protein is expressed, such as heart and lung. The invention further comprises a method of modulating apoptosis in a cell comprising an administration of an apoptosis-modulating effective amount of an agent which interferes with prion protein (PrP)-bax interaction.

Title: WO0249662A2: METHOD OF PROTECTING CELLS AGAINST APOPTOSIS AND ASSAYS TO IDENTIFY AGENTS WHICH MODULATE APOPTOSIS
Date:
Inventor: LEBLANC, Andrea; 1253 Marthe Mongrain, Chambly, Quebec J3L 5N4, Canada
BOUNHAR, Younes; 5014 Dornal, Montreal, Quebec H3W 1W2, Morocco
ZHANG, Yan; Apartment 22, 3330 Edouard Montpetit, Montreal, Quebec H3T 1K5, China
Assignee: 2002-06-27
Abstract: The present invention relates to a method of protecting neurons against bax-mediated apoptosis and assays to identify agents which modulate neuron apoptosis. The present invention further relates to apoptosis modulation in other tissues in which prion protein is expressed, such as heart and lung. The invention further comprises a method of modulating apoptosis in a cell comprising an administration of an apoptosis-modulating effective amount of an agent which interferes with prion protein (PrP)-bax interaction.

Title: WO0190191A3: ANTIBODIES CAPABLE TO SELECTIVELY DETECT PRION PrPSc ISOFORMS
Date: 2001-11-29
Inventor: CURIN-SERBEC, Vladka; Polanskova 44, 1000 Ljubljana, Republic of Slovenia

Assignee: BLOOD TRANSFUSION CENTRE OF SLOVENIA, Slajmerjeva 6, 1000 Ljubljana, Republic of Slovenia
Abstract: The present invention pertains to novel antibodies directed to the C-terminal part of the PrP isoform of prions. In particular, the present invention pertains to the use of such antibodies in the diagnosis of bovine spongiform encephalopathy (BSE) and the new variant form of Creutzfeld Jacobs Disease (vCJD). In addition, the present invention relates to the peptide recognized by the antibodies and its use in the immunization and/or treatment of BSE, CJD, vCJD and other TSE related diseases.

Title: EP1158003A1: Antibodies capable to selectively detect prion PrP Sc isoforms
Date: 2001-11-28
Inventor: Curin-Serbec, Dr. Blood Transfusion Ctr Slovenia;
Assignee: Blood Transfusion Centre of Slovenia
Abstract: The present invention pertains to novel antibodies directed to the C-terminal part of the PrPSc isoform of prions. In particular, the present invention pertains to the use of such antibodies in the diagnosis of bovine spongiform encephalopathy (BSE) and the new variant form of Creutzfeld Jacobs Disease (vCJD). In addition, the present invention relates to the peptide recognized by the antibodies and its use in the immunization and/or treatment of BSE, CJD, vCJD and other TSE related diseases.

Title: US6514707: Methods for detection of prion protein as an indication of transmissible spongiform encephalophathies
Date: 2003-02-04
Inventor: O'Rourke, Katherine I.; Pullman, WA
Knowles, Donald P.; Pullman, WA
Baszler, Timothy V.; Moscow, ID
Parish, Steven M.; Pullman, WA
Assignee: The United States of America as represented by the Secretary of Agriculture, Washington, DC
Washington State University Research Foundation, Pullman, WA
Abstract: Methods to detect prion or PrP-Sc protein as an indication of transmissible spongiform encephalopathies (TSEs), including preclinical detection of infected live animals, and postmortem detection methods, are described. In one aspect, the invention is directed to a non-invasive diagnostic assay using third eyelid-associated lymphoid tissue. In another aspect, the invention is directed to monoclonal antibodies that specifically bind a conserved epitope of PrP-Sc protein in fixed or frozen treated tissue.

Title: WO0134631A3: PEPTIDE ANALOGS AND MIMETICS SUITABLE FOR IN VIVO USE IN THE TREATMENT OF DISEASES ASSOCIATED WITH ABNORMAL PROTEIN FOLDING INTO AMYLOID, AMYLOID-LIKE DEPOSITS OR ß-SHEET RICH PATHOLOGICAL PRECURSOR THEREOF
Date: 2001-05-17
Inventor: SOTO-JARA, Claudio; 37 Chemin des Meandres, CH-1287 Laconnex, Switzerland
Assignee: AXONYX, INC., Suite 1400, 750 Lexington Avenue, New York, NY 10022, United States of America

Abstract: The present invention is an inhibitory peptide capable of inhibiting beta pleated sheet formation in amyloid beta -peptide. The inhibitory peptide is a beta sheet breaker peptide analog designed by chemical modification of beta sheet breaker peptide capable of inhibiting beta pleated sheet formation in amyloid beta -peptide. The present invention also includes an inhibitory peptide capable of inhibiting conformational changes in prion PrP protein associated with amyloidosis. The inhibitory peptide being a beta sheet breaker peptide analog designed by chemical modification of a beta sheet breaker peptide capable of inhibiting the conformational changes in prior PrP protein associated with amyloidosis. In addition, the present invention includes a peptide mimetic with the structure PMiA beta 5. In another embodiment, the peptide mimetic has the structure PMiPrP13. In yet another embodiment, the peptide mimetic has the structure PMiPrP5.

Title: CA2389041AA: PEPTIDE ANALOGS AND MIMETICS SUITABLE FOR IN VIVO USE IN THETREATMENT OF DISEASES ASSOCIATED WITH ABNORMAL PROTEIN FOLDING INTO AMYLOID, AMYLOID-LIKE DEPOSITS OR .BETA.-SHEET RICH PATHOLOGICAL PRECURSOR THEREOF
Date: 2001-05-17
Inventor: SOTO-JARA, CLAUDIO; Switzerland
Assignee: AXONYX, INC. United States of America
Abstract: The present invention is an inhibitory peptide capable of inhibiting ß pleated sheet formation in amyloid ß-peptide. The inhibitory peptide is a ßsheet breaker peptide analog designed by chemical modification of .bet a. sheet breaker peptide capable of inhibiting ß pleated sheet formation i n amyloid ß-peptide. The present invention also includes an inhibitory peptide capable of inhibiting conformational changes in prion PrP protein associated with amyloidosis. The inhibitory peptide being a ßsheet breaker peptide analog designed by chemical modification of a ßsheet breaker peptide capable of inhibiting the conformational changes in prior Pr P protein associated with amyloidosis. In addition, the present invention includes a peptide mimetic with the structure PMiA? . In another embodiment, the peptide mimetic has the structure PMiPrP13. In yet another embodiment, the peptide mimetic has the structure PMiPrP5.

Title: WO0105426A1: MONOCLONAL ANTIBODIES AND ANTIBODY COCKTAIL FOR DETECTION OF PRION PROTEIN AS AN INDICATION OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES
Date: 2001-01-25
Inventor: O'ROURKE, Katherine, I.; 705 West 1st Street, Albion, WA 99102, United States of America
Assignee: THE UNITED STATES OF AMERICA, as represented by THE SECRETARY OF AGRICULTURE, 1400 Independence Avenue SW, Washington, DC 20250-0302, United States of America
Abstract: Methods to detect prion or PrP-Sc protein as an indication of transmissible spongiform encephalopathies (TSEs) are described. In one aspect, the invention is directed to monoclonal antibodies that specifically bind a conserved epitope of prion proteins and use of the antibodies in immunoassays to detect PrP-Sc, in fixed or unfixed tissue, as an indication of the presence of TSE infection. In another aspect, the invention is directed to a monoclonal antibody

cocktail having the monoclonal antibody in combination with a second monoclonal antibody which specifically binds to a second conserved epitope of prion proteins. One or both monoclonal antibodies of the cocktail can recognize epitopes found in all mammalian species in which a natural TSE has been reported and in a number of closely related species. Thus, the antibody cocktail provides high sensitivity, defined specificity, and broad reactivity to PrP proteins in spite of interspecies and intraspecies variation of species such as ruminant livestock, cats, mink, humans, and non-human primates.

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